STUDIES IN THE BLOOD CYTOLOGY OF THE RABBIT

IV. Consecutive Neutrophile, Basophile, and Eosinophile Observations on Groups of Normal Rabbits

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(Received for publication, April 14, 1930)

The two preceding papers of this series have dealt with the consecutive observations on the erythrocytes and the hemoglobin content (1) and with the total white cells (2) of the peripheral blood of normal male rabbits in a series of experiments extending over long periods of time. In the present paper, the results pertaining to the neutrophile (pseudo-eosinophile), basophile, and eosinophile cells in the same five groups of animals are reported while in the succeeding paper (3), the lymphocytes and monocytes will be considered. Since this study was primarily undertaken for the purpose of obtaining information on the character of the general trends of cell levels over prolonged periods, the results are at present considered from the group rather than from the individual animal standpoint and the findings have been analyzed upon the basis of consecutive mean values obtained at weekly intervals.

Materials and Methods

These subjects have been fully discussed in connection with the erythrocyte and hemoglobin results (1) and need only be referred to here. The first four groups of rabbits comprised 10 animals each and the fifth group, 5 animals. The period of observation extended from October, 1927 to July, 1929 and the following numbers of weekly examinations were made: Group I, 35; Group II, 13; Group III, 8; Group IV, 29; Group V, 26. For the differential white cell counts, the supravital neutral red technic was used and 100 cells were counted in each specimen.

RESULTS

The results of these experiments dealing with the neutrophile, basophile, and eosinophile cell content of the peripheral blood of normal

TABLE I

Group I—10 Rabbits. Consecutive Values for Neutrophiles, Basophiles, and Eosinophiles

Date	Neutrophiles			Basophiles			Eosinophiles		
	Mean values	Standard deviation	Coefficient of variation	Mean values	Standard deviation	Coefficient of variation	Mean values	Standard deviation	Coefficient of variation
1927–28	*per cmm.*	*per cmm.*	*per cent*	*per cmm.*	*per cmm.*	*per cent*	*per cmm.*	*per cmm.*	*per cent*
Oct. 24*	4038 ± 279	1307	32.37	1276 ± 156	730	57.21	273 ± 43	201	73.63
Nov. 1	4340 ± 295	1383	31.87	791 ± 48	225	28.45	162 ± 12	56	34.57
Nov. 8**	3775 ± 256	1198	31.74	902 ± 85	398	44.12	271 ± 28	131	48.34
Nov. 15	3266 ± 173	811	24.83	1235 ± 128	602	48.74	400 ± 80	376	94.00
Nov. 22	5001 ± 388	1820	36.39	1045 ± 122	574	54.93	476 ± 87	408	85.71
Nov. 29	4470 ± 638	2992	66.94	813 ± 112	525	64.58	211 ± 31	143	67.77
Dec. 6	3877 ± 394	1848	47.67	842 ± 75	350	41.57	268 ± 29	135	50.37
Dec. 13	3774 ± 355	1663	44.06	774 ± 95	447	57.75	234 ± 40	185	79.06
Dec. 20	4905 ± 413	1934	39.43	1024 ± 99	465	45.41	322 ± 32	149	46.27
Dec. 27	4818 ± 265	1243	25.80	963 ± 124	579	60.12	202 ± 33	154	76.24
Jan. 3	4618 ± 253	1185	25.66	905 ± 72	336	37.13	288 ± 44	207	71.88
Jan. 10	4488 ± 365	1712	38.15	1138 ± 129	606	53.25	201 ± 30	142	70.65
Jan. 17	4566 ± 459	2151	47.11	1180 ± 161	755	63.98	154 ± 30	140	90.91
Jan. 24	4242 ± 294	1380	32.53	1373 ± 168	787	57.32	277 ± 52	244	88.09
Jan. 31	5725 ± 529	2481	43.34	6686 ± 157	738	107.58	180 ± 28	130	72.22
Feb. 7	4820 ± 472	2215	45.95	1358 ± 177	831	61.19	299 ± 40	189	63.21
Feb. 14	5688 ± 530	2485	43.69	763 ± 65	305	39.97	193 ± 37	173	89.64
Feb. 21	4544 ± 366	1715	37.74	1710 ± 113	529	30.94	290 ± 42	197	67.93
Feb. 28	6687 ± 883	4142	61.94	1111 ± 157	735	66.16	244 ± 46	215	88.11
Mar. 6	4790 ± 289	1356	28.31	1291 ± 233	1092	84.59	308 ± 45	210	68.18
Mar. 13	5208 ± 459	2151	41.30	787 ± 94	443	56.29	191 ± 40	186	97.38
Mar. 20	6653 ± 816	3826	57.51	707 ± 75	353	49.93	255 ± 65	305	119.61

Mar. 27	4662 ± 375	1756	37.67	1231 ± 115	538	43.70	170 ± 33	153	90.00
Apr. 3	4851 ± 367	1719	35.44	1046 ± 108	506	48.37	306 ± 33	154	50.33
Apr. 10	5793 ± 401	1880	32.45	1078 ± 127	597	55.38	400 ± 58	274	68.50
Apr. 17	5060 ± 359	1684	33.28	1108 ± 124	579	52.26	217 ± 23	109	50.23
Apr. 24	5256 ± 516	2419	46.02	1046 ± 92	429	41.01	220 ± 57	268	121.82
May 1	4851 ± 278	1304	26.88	1121 ± 69	321	28.64	159 ± 39	183	115.09
May 8	5055 ± 401	1880	37.19	1399 ± 173	813	58.11	190 ± 30	139	73.16
May 15	5111 ± 382	1791	35.04	1234 ± 107	501	40.60	416 ± 89	416	85.58
May 22	5901 ± 597	2797	47.40	1103 ± 130	609	55.21	213 ± 33	155	72.77
May 29	5269 ± 382	1789	33.95	981 ± 83	389	39.65	193 ± 29	135	69.95
June 5	5751 ± 361	1691	29.40	1462 ± 127	595	40.70	233 ± 50	232	99.57
June 12	5048 ± 334	1567	31.04	1339 ± 162	760	56.76	270 ± 28	133	49.26
June 19	5343 ± 425	1992	37.28	1364 ± 183	857	62.83	268 ± 42	197	73.51
Mean	4919 ± 268	2349	47.75	1091 ± 27	238	21.81	256 ± 9	76	29.69
Minimum	3266			686			154		
Maximum	6687			1710			476		

* October 24 and 26.

** November 4 and 9.

TABLE II

Group II—10 Rabbits. Consecutive Values for Neutrophiles, Basophiles, and Eosinophiles

Date	Neutrophiles			Basophiles			Eosinophiles		
	Mean values	Standard deviation	Coefficient of variation	Mean values	Standard deviation	Coefficient of variation	Mean values	Standard deviation	Coefficient of variation
1928	*per cmm.*	*per cmm.*	*per cent*	*per cmm.*	*per cmm.*	*per cent*	*per cmm.*	*per cmm.*	*per cent*
Mar. 29	3582 ± 284	1330	37.13	579 ± 42	197	34.02	139 ± 25	116	83.45
Apr. 3	3821 ± 220	1031	26.98	768 ± 88	411	53.52	155 ± 19	91	58.71
Apr. 10	3825 ± 340	1593	41.65	550 ± 55	258	46.91	140 ± 25	117	83.57
Apr. 17	4481 ± 320	1501	33.50	882 ± 94	442	50.11	277 ± 76	358	129.24
Apr. 24	4420 ± 403	1890	42.76	859 ± 110	520	60.54	152 ± 18	86	56.58
May 1	3123 ± 106	497	15.91	831 ± 108	505	60.77	150 ± 26	124	82.67
May 8	4111 ± 280	1311	31.89	871 ± 49	231	26.52	111 ± 21	96	86.49
May 15	3515 ± 359	1685	47.94	590 ± 65	306	51.86	180 ± 27	128	71.11
May 22	4920 ± 333	1559	31.69	954 ± 100	467	48.95	258 ± 33	155	60.08
May 29	4008 ± 350	1642	40.97	726 ± 88	411	56.61	113 ± 30	141	124.78
June 5	4348 ± 281	1319	30.34	1103 ± 84	392	35.54	118 ± 18	86	72.88
June 12	3653 ± 213	999	27.35	958 ± 113	528	55.11	111 ± 16	76	68.47
June 19	4632 ± 190	889	19.19	856 ± 109	510	59.58	175 ± 25	116	66.29
Mean	4034 ± 92	491	12.17	810 ± 29	157	19.38	160 ± 10	51	31.88
Minimum	3123			550			111		
Maximum	4920			1103			277		

TABLE III

Group III—10 Rabbits. Consecutive Values for Neutrophiles, Basophiles, and Eosinophiles

Date	Neutrophiles			Basophiles			Eosinophiles		
	Mean values	Standard deviation	Coefficient of variation	Mean values	Standard deviation	Coefficient of variation	Mean values	Standard deviation	Coefficient of variation
1928	*per cmm.*	*per cmm.*	*per cent*	*per cmm.*	*per cmm.*	*per cent*	*per cmm.*	*per cmm.*	*per cent*
Sept. 20	4777 ± 484	2270	47.52	437 ± 42	198	45.31	144 ± 47	221	153.47
Sept. 28	3002 ± 163	762	25.13	586 ± 50	232	39.59	179 ± 30	140	78.21
Oct. 10	3913 ± 467	2188	55.93	671 ± 84	395	58.87	215 ± 32	149	69.30
Oct. 19	2682 ± 263	1235	46.05	835 ± 66	308	36.89	157 ± 18	86	54.78
Nov. 2	3853 ± 281	1315	34.13	733 ± 64	301	41.06	168 ± 39	184	109.52
Nov. 9	3620 ± 213	1000	27.16	836 ± 102	477	57.06	257 ± 47	218	84.82
Nov. 16	3004 ± 261	1223	40.71	684 ± 57	267	39.04	252 ± 34	157	62.30
Nov. 22	3006 ± 249	1167	38.44	708 ± 69	321	45.34	178 ± 25	115	64.61
Mean	3482 ± 154	647	18.58	686 ± 29	122	17.78	194 ± 10	40	20.62
Minimum	2682			437			144		
Maximum	4777			836			257		

TABLE IV

Group IV—10 Rabbits. Consecutive Values for Neutrophiles, Basophiles, and Eosinophiles

Date	Neutrophiles			Basophiles			Eosinophiles		
	Mean values	Standard deviation	Coefficient of variation	Mean values	Standard deviation	Coefficient of variation	Mean values	Standard deviation	Coefficient of variation
1928–29	*per cmm.*	*per cmm.*	*per cent*	*per cmm.*	*per cmm.*	*per cent*	*per cmm.*	*per cmm.*	*per cent*
Nov. 27	3880 ± 246	1154	29.75	602 ± 86	401	66.67	111 ± 25	118	106.62
Dec. 4	3639 ± 286	1342	36.88	742 ± 96	450	60.60	73 ± 14	64	88.39
Dec. 11	3535 ± 280	1312	37.11	812 ± 55	257	31.59	120 ± 20	96	80.06
Dec. 18	2938 ± 158	742	25.23	668 ± 77	360	53.87	64 ± 14	64	99.56
Dec. 26	3440 ± 258	1208	35.12	670 ± 74	349	52.15	82 ± 18	86	104.89
Jan. 2	3530 ± 174	816	23.12	735 ± 63	297	40.39	55 ± 15	71	128.90
Jan. 8	2870 ± 279	1310	45.64	608 ± 63	296	48.61	117 ± 28	133	113.73
Jan. 15	3396 ± 172	808	23.78	573 ± 64	300	52.31	117 ± 17	81	69.22
Jan. 22	3221 ± 255	1195	37.11	758 ± 77	362	47.81	123 ± 21	100	81.44
Jan. 29	4442 ± 292	1371	30.87	965 ± 83	387	40.14	103 ± 20	95	92.19
Feb. 5	3481 ± 331	1551	44.55	552 ± 36	167	30.16	83 ± 18	84	100.69
Feb. 13	5170 ± 378	1771	34.27	632 ± 78	367	58.03	177 ± 29	137	77.51
Feb. 19	6085 ± 328	1536	25.24	741 ± 71	334	45.02	224 ± 61	285	127.40
Feb. 26	5403 ± 360	1687	31.21	850 ± 127	594	69.87	112 ± 22	101	90.06
Mar. 12	4251 ± 215	1010	23.75	408 ± 53	247	60.53	72 ± 16	77	107.43
Mar. 19	5544 ± 426	1996	36.00	514 ± 30	142	27.52	201 ± 30	139	69.35
Mar. 26	5483 ± 351	1644	29.97	400 ± 44	204	51.00	44 ± 10	47	105.90
Apr. 2	5342 ± 384	1799	33.67	612 ± 58	273	44.53	105 ± 25	115	109.60
Apr. 9	5160 ± 133	625	12.11	377 ± 31	147	39.08	149 ± 26	124	83.08
Apr. 16	5396 ± 314	1470	27.24	434 ± 56	262	60.36	55 ± 16	76	137.40
Apr. 23	5609 ± 218	1023	18.23	505 ± 57	267	52.90	132 ± 39	185	140.10
Apr. 30	5384 ± 220	1031	19.14	474 ± 66	311	65.54	54 ± 15	70	128.90

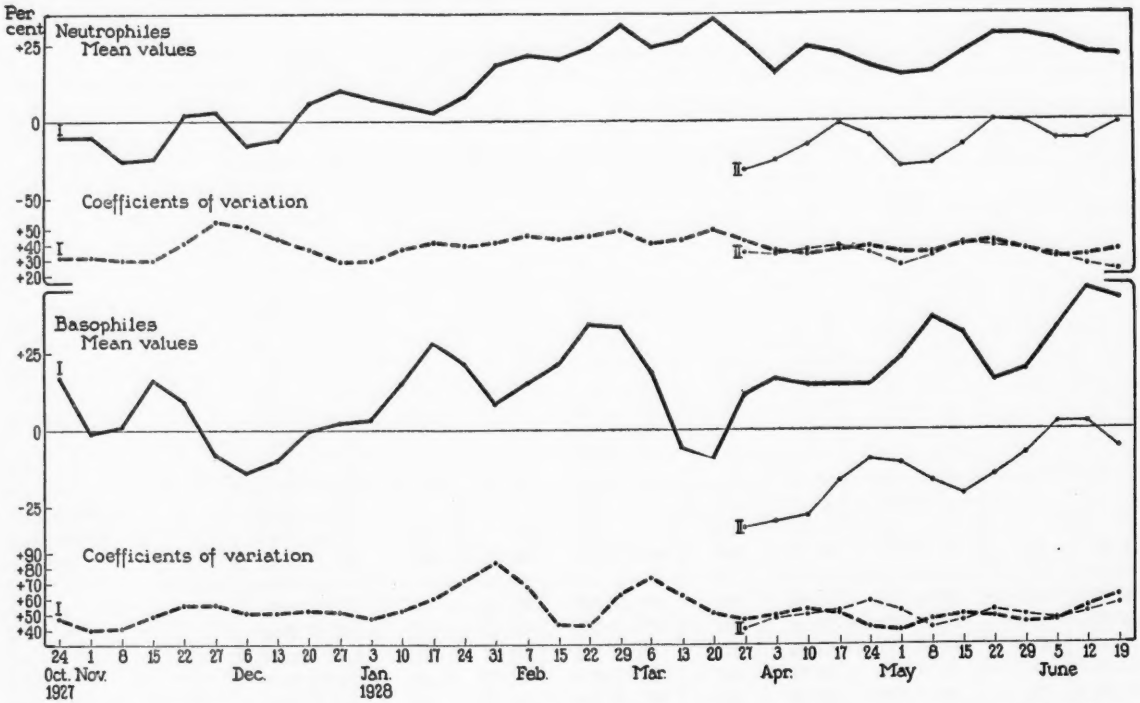
May 7	5434 ± 332	1557	28.66	479 ± 60	280	58.41	153 ± 28	130	84.64
May 14	4380 ± 199	883	20.15	490 ± 104	464	94.59	106 ± 8	35	117.63
May 21	4826 ± 248	1101	22.80	388 ± 75	332	85.47	26 ± 12	55	210.00
May 28	4769 ± 231	1026	21.51	564 ± 100	443	78.58	87 ± 27	122	139.65
June 4	4123 ± 263	1170	28.37	386 ± 52	231	59.85	36 ± 13	56	155.27
June 11	4085 ± 209	930	22.77	455 ± 68	301	66.04	37 ± 9	42	114.59
June 18	3464 ± 164	688	19.86	343 ± 50	211	61.57	76 ± 17	71	93.00
Mean	4423 ± 117	933	21.09	577 ± 20	157	27.20	100 ± 6	48	48.01
Minimum	2870			343			26		
Maximum	6085			965			224		

TABLE V

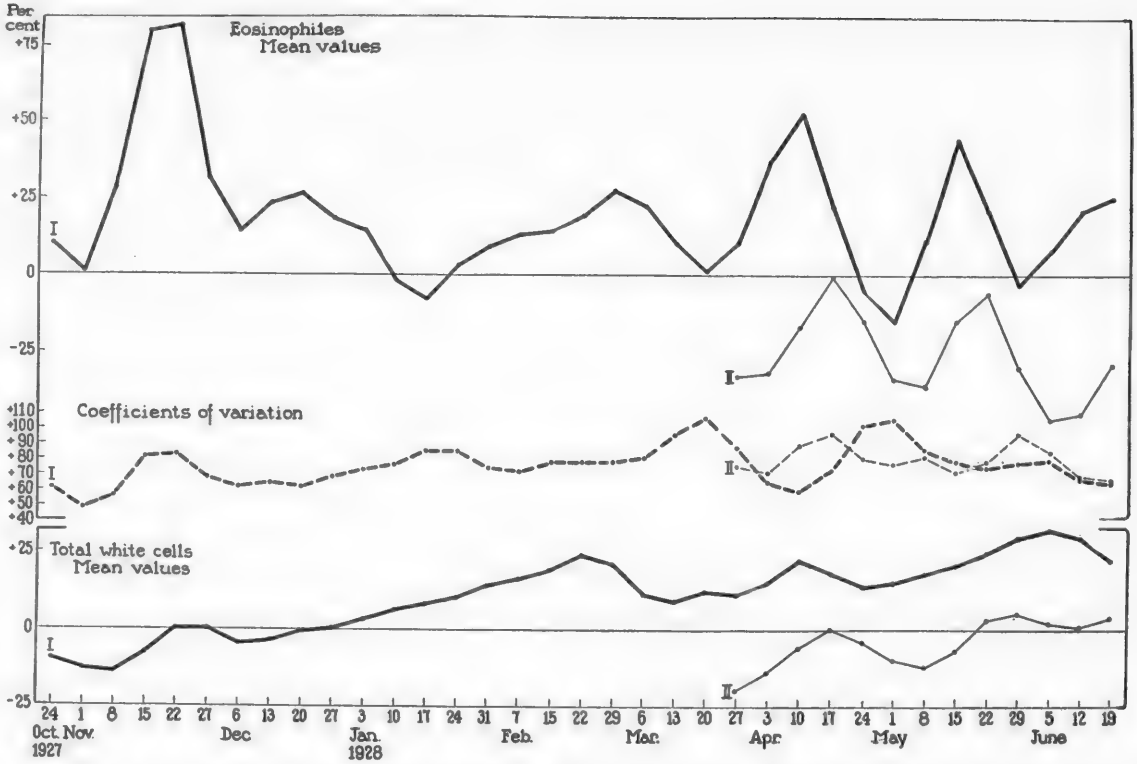
Group V—5 Rabbits. Consecutive Values for Neutrophiles, Basophiles, and Eosinophiles

Date	Neutrophiles			Basophiles			Eosinophiles		
	Mean values	Standard deviation	Coefficient of variation	Mean values	Standard deviation	Coefficient of variation	Mean values	Standard deviation	Coefficient of variation
1928–29	*per cmm.*	*per cmm.*	*per cent*	*per cmm.*	*per cmm.*	*per cent*	*per cmm.*	*per cmm.*	*per cent*
Dec. 21	2062 ± 191	632	30.65	290 ± 57	188	64.83	17 ± 6	21	123.53
Dec. 31	1892 ± 197	654	34.57	498 ± 66	220	44.18	19 ± 7	24	126.32
Jan. 3	2815 ± 326	1081	38.40	614 ± 41	135	21.99	117 ± 32	106	90.60
Jan. 10	1915 ± 251	833	43.50	525 ± 46	151	28.76	74 ± 15	51	68.92
Jan. 24	2014 ± 115	382	18.97	381 ± 38	127	33.33	36 ± 13	44	122.22
Jan. 31	2097 ± 272	902	43.01	642 ± 171	568	88.47	73 ± 13	42	57.53
Feb. 7	1994 ± 162	537	26.97	333 ± 59	196	58.86	124 ± 39	130	104.84
Feb. 15	2999 ± 392	1298	43.28	529 ± 92	304	57.47	121 ± 26	85	70.25
Feb. 21	3677 ± 176	584	15.88	461 ± 29	96	20.82	156 ± 35	117	75.00
Mar. 1	2850 ± 84	279	9.79	507 ± 88	292	57.59	190 ± 39	130	68.42
Mar. 8	3320 ± 145	480	14.46	454 ± 20	65	14.32	147 ± 21	68	46.26
Mar. 15	2680 ± 135	449	16.75	656 ± 46	224	34.15	226 ± 53	175	77.43
Mar. 22	3012 ± 140	465	15.44	363 ± 61	202	55.65	219 ± 38	127	57.99
Mar. 29	3650 ± 283	937	25.67	484 ± 118	390	80.58	189 ± 41	136	71.96
Apr. 5	3669 ± 207	685	18.67	512 ± 43	144	28.13	180 ± 20	67	37.22
Apr. 12	3094 ± 186	617	19.94	382 ± 62	204	53.40	181 ± 35	115	63.54
Apr. 19	4171 ± 246	817	19.59	410 ± 32	106	25.85	153 ± 36	118	77.12
Apr. 26	3715 ± 213	705	18.98	528 ± 76	253	47.92	98 ± 24	80	81.63
May 3	3612 ± 294	975	26.99	615 ± 57	190	30.89	127 ± 39	130	102.36
May 10	3171 ± 240	797	25.13	458 ± 64	211	46.07	208 ± 67	221	106.25
May 17	3774 ± 322	1068	28.30	461 ± 40	132	28.63	115 ± 29	95	82.61

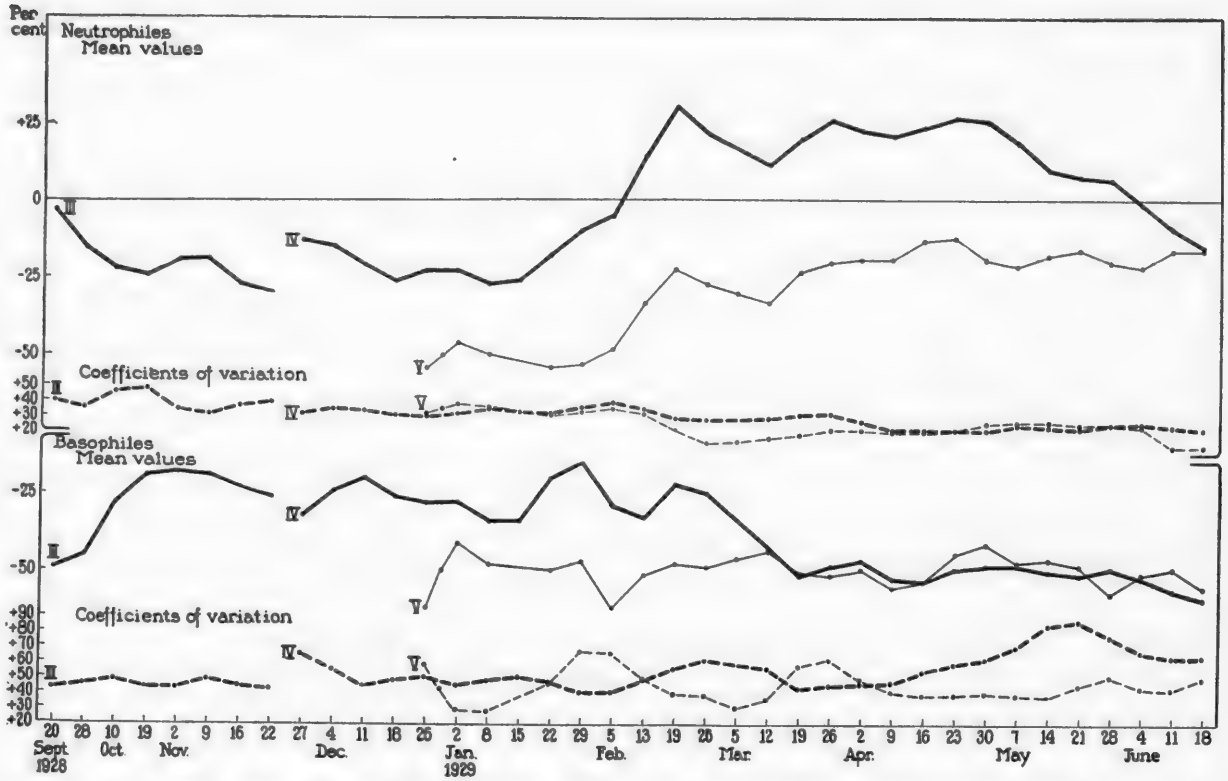
May 24	3604 ± 239	791	21.95	634 ± 86	284	44.79	126 ± 16	54	42.86
May 31	3616 ± 276	916	25.33	212 ± 36	120	56.60	37 ± 14	47	12.70
June 7	3045 ± 215	714	23.45	546 ± 66	220	40.29	126 ± 35	115	91.27
June 14	4005 ± 227	752	18.78	492 ± 54	178	36.18	127 ± 41	135	106.30
June 21	3519 ± 211	698	19.84	382 ± 65	214	56.02	194 ± 50	164	84.54
Mean	3076 ± 92	692	22.50	476 ± 14	109	22.90	130 ± 8	59	45.38
Minimum	1892			212			17		
Maximum	4171			652			226		



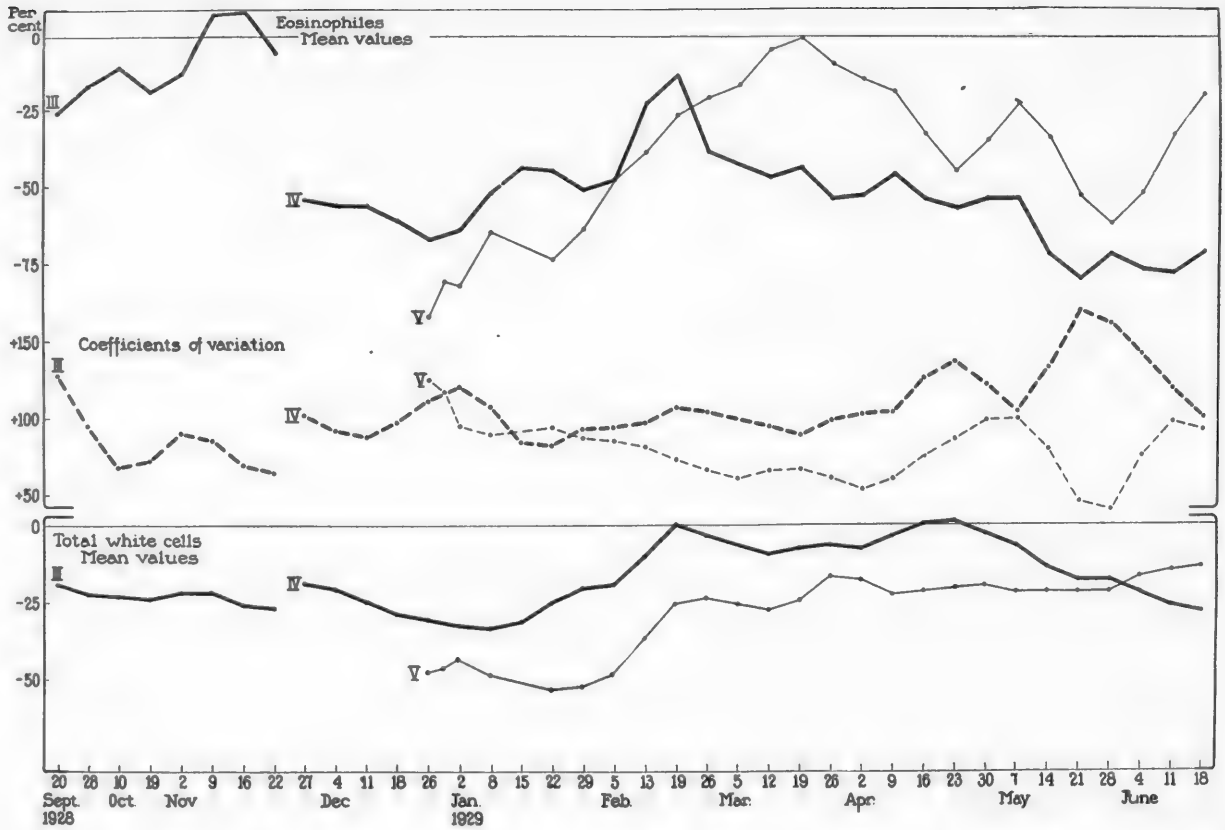
TEXT-FIG. 1. Mean values for consecutive neutrophile and basophile determinations as percentage deviations from standard values. 1927–28.



TEXT-FIG. 2. Mean values for consecutive eosinophile and total white blood cell determinations as percentage deviations from standard values. 1927–28.



TEXT-FIG. 3. Mean values for consecutive neutrophile and basophile determinations as percentage deviations from standard values. 1928–29.



TEXT-FIG. 4. Mean values for consecutive eosinophile and total white blood cell determinations as percentage deviations from standard values. 1928–29.

rabbits examined over long periods of time are presented in the form of consecutive weekly mean values for each group of animals (Tables I to V). In addition, the probable errors of the means, the standard deviations, and the coefficients of variation are given. The series of curves in Text-figs. 1 to 4 representing the mean values, are drawn from the percentage deviations of the smoothed means from the following standard values (1, 4): neutrophiles, 4340 cells per cubic millimeter; basophiles, 950 cells per cubic millimeter; eosinophiles, 215 cells per cubic millimeter. The curves of the smoothed coefficients of variation of the means are also given in Text-figs. 1 to 4. These charts also include curves which represent the total white cell means in terms of the percentage deviations of the smoothed means from a standard value of 9560 cells per cubic millimeter, in order that comparisons may readily be made between the results on the various granular leucocytes and those on the total white count without referring to the preceding paper (2) in which the latter observations are discussed.

DISCUSSION AND SUMMARY

For convenience of discussion, the results of these experiments dealing with consecutive neutrophile, basophile, and eosinophile counts will be considered in the chronological order of the five groups of rabbits studied.

Group I was examined from October 24, 1927 to June 21, 1928 (Table I). It will be seen by referring to Text-fig. 1, that the mean numbers of neutrophiles had increased approximately 30 per cent by the second half of the experiment and that thereafter, this higher level was generally maintained. In October, November, and December, the general level of the curve fluctuates slightly below and above the standard value of 4340 cells per cubic millimeter represented by the base line. During January, February, and March, a decided upward trend is noted and thereafter to the last of June, a slightly lower level is observed.

The mean numbers of basophiles in Group I (Table I) were also increased during the experiment, but as will be seen from the curve in Text-fig. 1, the change was accomplished in a much more irregular fashion than in the case of the neutrophiles. During October and November, the general trend of the curve is downward from an

initial level above the base line (950 cells per cubic millimeter); in December, January, and February, the curve describes an irregular but marked rise which is followed by an abrupt fall to the November level; at the end of March and continuing through April, May, and June, there is a second rise which carries the curve to the highest level observed (55 per cent above the base line). During these periods of increasing value, there were two interruptions, the first occurring the end of January and the second in May.

In the case of the eosinophiles of Group I (Table I) pronounced variations in mean numbers were observed, as is shown by the curve in Text-fig. 2. Throughout November and again in April and May, large numbers of these cells were observed which account for the three prominent and abrupt peaks of the curve. Following each marked increase, the mean values sank to a comparatively low level, but on the whole, the means throughout the experiment were higher than the standard value of 215 cells per cubic millimeter.

Comparing the general changes in the mean values of the three classes of granular leucocytes with that of the total white cells (Text-fig. 2), it will be noted that during the first half of the experiment (October to March) increasing values were observed in the total white cells, the neutrophiles, and the basophiles (after November) which are indicated by the upward direction of their curves. On the other hand, this feature was not found in the eosinophile results until the January and February observations. During the latter part of February and throughout March, lower levels prevailed in the total white cell, the basophile, and the eosinophile means but in the case of the neutrophiles, the values although comparatively irregular, were on the whole not diminished. In the last period of the experiment, the trend of the total white cells was again upward, a movement characteristic also of the basophiles and to a slight extent of the neutrophiles. The later eosinophile means which showed no consistent trend in one direction or another were characterized by a series of abrupt and pronounced swings. The high points of these fluctuations (February 29, April 10, and May 15), however, occurred at the time of high neutrophile values and somewhat analogous findings were observed in the earlier weeks of the experiment (November 22 and December 20).

The coefficients of variation of the neutrophile means of Group I (Table I) are fairly uniform although their order of magnitude is high.

The curve of the smoothed values (Text-fig. 1) shows variations within a 10 per cent range at the general level of 30 to 40 per cent except in November and December when the abrupt alteration of mean values is accompanied by a sharp upward turn of the coefficient curve. In the case of the basophiles (Table I) the curve of the smoothed coefficients of variation (Text-fig. 1) is fairly regular at the 40 to 50 per cent level except for two major upward swings the end of January and the first of March which accompany not increased, but diminished mean values. In the last third of the experiment, during which the basophile means rose to the highest figure observed, the coefficients of variation are comparatively uniform. The coefficients of variation of the eosinophile means (Table I) are of a very high order of magnitude, the curve of the smoothed values ranging from 47 to 107 per cent. The highest points on the curve correspond to the period of extremely high mean values in November but to the low mean values in January, March, and May which immediately succeeded abruptly increased counts. During these phases of augmented mean values, the coefficients of variation are smaller so that the curve is directed downward.

Group II was examined from March 29 to June 19, 1928 (Table II). The curves illustrating the neutrophile, the basophile, and the eosinophile means as percentage deviations from standard values (Text-figs. 1 and 2) show a striking resemblance in general contour to the curves of Group I for this period as is also the case with the total white cells (Text-fig. 2); the levels of the means of Group II, however, are considerably lower. The two prominent peaks of the eosinophile curve in April and May, occurring almost at the same time as those of Group I, are worthy of special note in view of the comparatively small numbers of these cells.

The coefficients of variation of these three cell means of Group II (Table II) are of the same order of magnitude as those of Group I. The curves of the smoothed values (Text-figs. 1 and 2) are similar to those of Group I except in the case of the eosinophiles in which one of the two highest points corresponds to and the other directly follows, high mean values.

Group III was examined from September 20 to November 22, 1928 (Table III). The neutrophile, basophile, and eosinophile mean values were generally lower than those of Group I and were more comparable to those of Group II, as may be seen by comparing the respective curves in Text-figs. 1, 2, 3, and 4. In the case of the neutro-

phile curve, there is a prevailing downward trend which is described in a fairly regular fashion while the direction of the basophile and eosinophile curves is upward. The curve for the total white cells, on the other hand, is almost a straight line (Text-fig. 4).

The coefficients of variation of these means (Table III) are of similar orders of magnitude to those of Groups I and II, but contrary to what was found in Group I, the curve of the smoothed neutrophile values shows more irregularities than that of the basophiles (Text-fig. 3). The highest point of the eosinophile coefficient curve (Text-fig. 4) corresponds to the smallest mean value, a feature which was generally characteristic of Group I.

Group IV was examined from November 27, 1928 to June 18, 1929 (Table IV). On the whole, the mean values for the neutrophile, the basophile, and the eosinophile cells were generally smaller than those of Group I. In the case of the neutrophiles (Text-fig. 3), low initial levels prevailed until the middle of January, at which time a pronounced increase occurred and a level of approximately 25 per cent above the standard value was maintained until the end of April, after which progressively smaller values were found. In general form, the neutrophile curve resembles that of the total white cells. The highest basophile values, on the other hand, were observed in the first half of the experiment as is shown by the curve in Text-fig. 3; in the second half, the curve occupies a uniform position 25 per cent lower than its first level. The two small peaks in the basophile curve at the end of January and toward the end of February occur at the time of the pronounced upward swing of the neutrophile curve. The trend of the first part of the eosinophile curve (Text-fig. 4) is in a rising direction culminating abruptly in a sharp peak the latter part of February. From then onward to the end of the experiment, the curve describes an irregular fall and ends at a lower level than that of the initial observations.

The coefficients of variation of the neutrophile, basophile, and eosinophile means of Group IV (Table IV) are comparable in their orders of magnitude to those of the other groups. From the curves of the smoothed values, it will be seen that that of the neutrophiles was more uniform than that of the basophiles (Text-fig. 3) which is the case with Groups I and II but not with Group III. It should be noted that the marked changes in the level of the neutrophile mean values are not accompanied by pronounced fluctuations of the coefficient

curve. The general level of the basophile coefficient curve is fairly well maintained until April and May when an upward swing occurs although no accompanying change in the curve representing the mean values is seen. In the case of the eosinophiles (Text-fig. 4), the coefficient curve is fairly regular until April and May when there are two abrupt upward fluctuations. These changes occur during diminishing mean values and the second high coefficient peak follows a marked drop in the mean values during the first part of May.

Group V was examined from December 29, 1928 to June 21, 1929 (Table V). The curves representing the mean values of the neutrophile, basophile, and eosinophile cells (Text-figs. 3 and 4) are similar in general outline to those of Group IV examined during the same months. In the case of the neutrophiles, however, much lower values prevailed until the end of the experiment. A comparison of the neutrophile and total white cell curves brings out the fact of their similarity with respect to general form as was also found with the other groups. The basophile curve for Group V which is relatively stable at a level of 50 per cent below the standard value shows the smallest fluctuations of any of the basophile curves. In many places, however, those which do occur correspond to similar alterations in the curve of the Group IV basophiles. In the case of the eosinophiles, the curves of the two groups bear a general resemblance to each other but the swings of the Group V curve are less pronounced, the summit of its marked rise in the middle of the observation period occurs somewhat later, and its second half occupies a higher level.

The coefficients of variation of the neutrophile means of Group V (Table V) are generally comparable to those of Group IV (Text-fig. 3); those of the basophiles (Table V) are on the whole somewhat smaller (Text-fig. 3). The coefficients of the eosinophile means of Group V are generally smaller than those of Group IV (Table IV). In the curve of the smoothed values (Text-fig. 4) it should be noted that comparatively low levels prevail during February and March in which months the mean values were progressively high. In its latter portion, the curve described two abrupt upward swings, the first of which corresponds to decreasing and the second to increasing mean values.

There are certain features of these results obtained from the observations of many weeks to which attention may now be especially directed. In the first place, the neutrophiles, as would be expected in view of the relative number of these cells, influence the total white cell count to the extent that the curve representing the consecutive

smoothed mean values of the one usually resembles quite closely the curve of the other.

In the experiments carried out for the longer periods, Groups I and II in 1927–28 and Groups IV and V in 1928–29, an increase in the neutrophile mean values occurred in the late winter and early spring months but during May and June, lower or stationary levels prevailed. In Group III examined during the autumn of 1928, the trend was in the direction of smaller values, a finding which is in accord with the low values of the other groups in October, November, and December of both years. The results pertaining to the basophiles differed in the two years. In 1927–28, there was a general increase in mean values which continued through the spring months; in the second year, the trend was toward smaller values. The rising basophile values of Group III in the fall of 1928 were in accord with the early findings of Group IV, the first examination of which followed the last observations of Group III. In the case of the eosinophiles, the results were more irregular. Their most prominent feature was the abrupt and very pronounced increase and decrease of mean values occurring in November of 1927 and in the late winter and spring months of 1928 and 1929.

That the variations in the general levels of the neutrophile, the basophile, and the eosinophile mean values observed in these experiments have a statistical significance is shown by the following examples of the ratios of the differences of certain means to their probable errors:

Group number	Neutrophiles			Basophiles			Eosinophiles		
	Dates of mean values		Ratio	Dates of mean values		Ratio	Dates of mean values		Ratio
I	Nov. 15	June 5	6.21	Nov. 1	June 19	3.03	Mar. 13	Apr. 10	2.99
	Nov. 15	Jan. 31	4.48	Jan. 31	Feb. 21	5.28	Apr. 10	May 1	3.44
II	Mar. 29	June 19	3.07	Mar. 29	June 5	5.57	May 8	May 22	3.77
	May 1	May 22	5.15	May 15	June 5	4.84	May 15	June 12	3.97
III	Sept. 20	Oct. 19	3.81	Sept. 20	Oct. 19	5.10	Sept. 20	Nov. 9	1.69
IV	Jan. 2	Feb. 19	6.89	Nov. 27	Jan. 29	3.04	Jan. 2	Feb. 19	2.68
	Apr. 23	June 18	7.89	Jan. 29	Mar. 26	6.73	Feb. 13	June 11	4.67
V	Dec. 29	June 21	5.12	Dec. 29	June 21	2.94	Dec. 29	Mar. 15	3.94
	Jan. 10	Feb. 21	5.76	Jan. 3	Feb. 7	3.90	Mar. 15	Apr. 26	2.21

In general, it was found that in two groups of rabbits examined during the same months, the variations in the mean levels of the three classes of granular leucocytes were similar both as regards the direction of the change and the time at which it occurred. This group resemblance was particularly striking in the case of the marked fluctuations of the eosinophiles but it was also characteristic of the neutrophiles and the basophiles. The results of the two years differed in that the general level of the neutrophile, the basophile, and the eosinophile mean values was higher in the groups examined in 1927–28 than in those observed in 1928–29. Both features of group resemblance and the yearly difference of level were also characteristic of the results pertaining to the red blood cells and the hemoglobin content (1) and the total white, the granular, and the non-granular cells (2). In the case of the total white count and its granular and non-granular components, the period of greatest irregularity of mean values occurred in the late winter and spring months of both years. This was true of the neutrophiles in the second year but to a less extent in the first year. The basophile values were very variable throughout the first year while in the second, the greatest irregularities occurred in the winter months. The fluctuations of the eosinophiles were most pronounced in the late winter and spring months of both years.

In the majority of cases, the physical condition of the rabbits throughout the periods of observation was excellent and gains in body weight were the rule. There were, however, a number of instances of clinical snuffles of slight or moderate degree as well as several cases of ear canker. The effect of these conditions upon the blood count will subsequently be taken up in detail when the results of these and other experiments are considered from the standpoint of the individual animal and of a comparison with snuffles free stock; at present the subject can only be referred to briefly. There were 2 deaths among the 45 rabbits of these experiments, occurring in Group IV; both animals had had snuffles and in one, a chronic nephritis was found at postmortem examination. It appeared that the occurrence of a muco-purulent nasal discharge was most frequently associated with an increase of neutrophiles and in certain instances, of lymphocytes, while in a few cases, higher monocyte values were observed. The persistence of a nasal discharge was not, however,

necessarily accompanied by continued high counts. On the other hand, increased counts were observed in rabbits in which no intercurrent infection could be demonstrated by repeated clinical examination and in addition, comparable fluctuations of cell levels were found in both "normal" rabbits and in those with slight or moderate snuffles. The effect of ear canker upon the blood count was not clearly defined. In certain animals, its occurrence coincided with a transitory increase of the neutrophile and lymphocyte cells, while in others, no characteristic alteration of cell levels was observed. Although these conditions of intercurrent infection undoubtedly had an effect upon the blood count, nevertheless, they do not appear to be a factor of major importance with respect to the character of the general variations in cell levels observed over long periods of time from the standpoint of the consecutive mean values of these animal groups.

CONCLUSIONS

Consecutive weekly observations on the neutrophile, the basophile, and the eosinophile counts of the peripheral blood were made on 5 groups of normal rabbits, a total of 45 animals, during a period of 20 months from October, 1927 to July, 1929. Individual groups were examined 8 to 35 weeks.

In the case of the 4 groups followed 13 to 35 weeks, the general trend of the neutrophile cells was towards increased mean values; with the group followed 8 weeks, decreasing values were found. An increase in the mean values of the basophile cells was observed in the two groups of rabbits followed in 1927–28; in the groups of 1928–29, the mean values decreased. The mean values of the eosinophile cells showed no definite trends but the findings were characterized by abrupt and marked fluctuations.

The periods of greatest irregularity in mean neutrophile and eosinophile values occurred in the fall and the late winter and spring months of both years, but in the case of the basophiles, the irregularities were distributed throughout the first year and occurred chiefly in the winter months of the second year.

The major trends and many of the minor fluctuations as well which were observed in the mean cell values of one group of rabbits were also generally seen in another group examined during the same months.

The general levels of the neutrophile, the basophile, and the eosinophile mean values in the groups examined during 1927–28 were higher than in the groups of 1928–29.

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STUDIES IN THE BLOOD CYTOLOGY OF THE RABBIT

V. Consecutive Lymphocyte and Monocyte Observations on Groups of Normal Rabbits

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(Received for publication, April 14, 1930)

Previous papers of this series have contained the results of repeated blood examinations on 5 groups of normal male rabbits with respect to the erythrocyte and hemoglobin determinations (1), the total white cell contents (2), and the neutrophile (pseudo-eosinophile), the basophile, and the eosinophile cell counts (3). The purpose of these experiments was to obtain information on the general character or trend of the spontaneous variations described by the cellular constituents of the peripheral blood over prolonged periods of time, and the data obtained have, in each instance, been presented on the basis of the consecutive weekly mean values of each group. In the present paper, the results on the non-granular cells—the lymphocytes and the monocytes— are reported.

Materials and Methods

A description of the materials and methods employed in these experiments and of the method of analyzing the results obtained has been given in the paper dealing with the observations on the erythrocytes and hemoglobin (1). Suffice it to say here that the period of time covered by the experiments extended from October, 1927 to July, 1929, and in the majority of cases, the blood was examined at weekly intervals. The numbers of examinations were: Group I, 35; Group II, 13; Group III, 8; Group IV, 29; Group V, 26. Each of the first 4 groups comprised 10 and the last, 5 normal male rabbits. The supravital neutral red technic was used in making the differential white cell counts and 100 cells were counted in each specimen.

RESULTS

The consecutive lymphocyte and monocyte determinations on 5 groups of normal rabbits are given in Tables I to V in the form of mean

TABLE I

Group I—10 Rabbits. Consecutive Values for Lymphocytes and Monocytes

Date	Lymphocytes			Monocytes		
	Mean values	Standard deviation	Coefficient of variation	Mean values	Standard deviation	Coefficient of variation
1927–28	*per cmm.*	*per cmm.*	*per cent*	*per cmm.*	*per cmm.*	*per cent*
Oct. 24*	2253 ± 185	867	25.97	987 ± 83	388	39.31
Nov. 1	1861 ± 92	431	23.16	1092 ± 139	650	59.52
Nov. 8**	1929 ± 165	772	40.02	1153 ± 104	487	42.24
Nov. 15	2560 ± 105	491	19.18	1085 ± 83	389	35.85
Nov. 22	2005 ± 169	794	39.60	1409 ± 185	869	61.67
Nov. 29	2984 ± 210	983	32.94	1209 ± 174	817	67.63
Dec. 6	2781 ± 238	1117	40.17	1073 ± 114	533	49.67
Dec. 13	3171 ± 399	1871	59.00	1178 ± 127	596	50.59
Dec. 20	2209 ± 166	778	35.22	1231 ± 127	595	48.33
Dec. 27	2554 ± 197	922	36.10	864 ± 77	362	41.90
Jan. 3	2945 ± 333	1559	52.94	1045 ± 52	245	23.44
Jan. 10	3425 ± 256	1202	35.09	967 ± 73	341	35.26
Jan. 17	3429 ± 352	1648	49.11	1025 ± 102	480	46.83
Jan. 24	3511 ± 285	1336	38.05	967 ± 58	270	27.92
Jan. 31	3444 ± 284	1332	38.68	1023 ± 81	380	37.15
Feb. 7	3501 ± 263	1235	35.28	1100 ± 106	497	45.18
Feb. 14	3070 ± 225	1056	34.40	1201 ± 134	627	52.21
Feb. 21	4671 ± 496	2324	49.75	1218 ± 148	693	56.90
Feb. 28	2507 ± 151	708	28.24	1145 ± 101	474	41.40
Mar. 6	2934 ± 183	859	29.28	1117 ± 76	354	31.69
Mar. 13	2532 ± 221	1037	40.96	1183 ± 138	648	54.78
Mar. 20	2563 ± 166	779	30.39	1084 ± 83	389	35.89
Mar. 27	3044 ± 217	1015	33.34	1112 ± 104	488	43.88
Apr. 3	3322 ± 335	1571	47.29	1181 ± 127	595	50.38
Apr. 10	3966 ± 337	1578	39.79	1027 ± 123	576	56.09
Apr. 17	3734 ± 245	1149	30.77	1107 ± 121	569	51.40
Apr. 24	3073 ± 288	1352	44.00	933 ± 134	627	67.20
May 1	4165 ± 316	1483	35.61	933 ± 93	438	46.95
May 8	3358 ± 251	1178	35.08	1092 ± 87	407	37.27
May 15	3775 ± 190	891	23.60	1071 ± 100	468	43.70
May 22	3624 ± 169	792	21.85	1245 ± 110	516	41.45
May 29	4182 ± 324	1521	36.37	1410 ± 294	1377	97.66
June 5	4602 ± 205	959	20.84	1543 ± 169	790	51.20
June 12	4455 ± 473	2219	49.81	1270 ± 117	546	42.99
June 19	3376 ± 292	1371	40.61	1106 ± 114	536	48.46
Mean	3186 ± 83	731	22.94	1125 ± 16	139	12.36
Minimum	1861			864		
Maximum	4671			1543		

* October 24 and 26.

** November 4 and 9.

TABLE II

Group II—10 Rabbits. Consecutive Values for Lymphocytes and Monocytes

Date	Lymphocytes			Monocytes		
	Mean values	Standard deviation	Coefficient of variation	Mean values	Standard deviation	Coefficient of variation
1928	*per cmm.*	*per cmm.*	*per cent*	*per cmm.*	*per cmm.*	*per cent*
Mar. 29	2210 ± 173	809	36.61	573 ± 35	165	28.80
Apr. 3	3313 ± 262	1227	37.04	677 ± 101	474	70.01
Apr. 10	3073 ± 287	1347	43.83	844 ± 89	416	49.29
Apr. 17	3429 ± 286	1342	39.13	1076 ± 154	724	67.29
Apr. 24	3067 ± 203	950	30.97	853 ± 102	476	55.80
May 1	3257 ± 231	1081	33.19	680 ± 64	299	43.97
May 8	3215 ± 216	1012	31.48	777 ± 83	388	49.94
May 15	2605 ± 247	1158	44.45	721 ± 126	590	81.83
May 22	3960 ± 284	1331	33.61	1028 ± 101	475	46.21
May 29	3758 ± 365	1713	45.58	843 ± 97	455	53.97
June 5	3864 ± 252	1180	30.54	796 ± 70	329	41.33
June 12	3236 ± 244	1144	35.35	1063 ± 110	517	48.64
June 19	3526 ± 222	1040	29.50	1166 ± 66	310	26.59
Mean	3270 ± 87	465	14.22	854 ± 32	173	20.26
Minimum	2210			573		
Maximum	3960			1166		

TABLE III

Group III—10 Rabbits. Consecutive Values for Lymphocytes and Monocytes

Date	Lymphocytes			Monocytes		
	Mean values	Standard deviation	Coefficient of variation	Mean values	Standard deviation	Coefficient of variation
1928	*per cmm.*	*per cmm.*	*per cent*	*per cmm.*	*per cmm.*	*per cent*
Sept. 20	1971 ± 268	1254	63.62	712 ± 113	529	74.30
Sept. 28	2454 ± 287	1345	54.81	780 ± 88	414	53.08
Oct. 10	2178 ± 82	385	17.68	739 ± 80	373	50.47
Oct. 19	2497 ± 142	667	26.71	813 ± 103	482	59.29
Nov. 2	2009 ± 167	784	39.02	797 ± 101	472	59.22
Nov. 9	2352 ± 151	708	30.10	820 ± 64	300	36.59
Nov. 16	1883 ± 110	516	27.40	733 ± 89	415	53.69
Nov. 22	2348 ± 181	850	36.20	895 ± 112	525	58.66
Mean	2212 ± 53	220	9.95	786 ± 13	55	7.00
Minimum	1883			712		
Maximum	2497			895		

values, together with the probable errors of the means, the standard deviations, and the coefficients of variation. The curves in Text-figs.

TABLE IV

Group IV—10 Rabbits. Consecutive Values for Lymphocytes and Monocytes

Date	Lymphocytes			Monocytes		
	Mean values	Standard deviation	Coefficient of variation	Mean values	Standard deviation	Coefficient of variation
1928–29	*per cmm.*	*per cmm.*	*per cent*	*per cmm.*	*per cmm.*	*per cent*
Nov. 27	2616 ± 173	812	31.02	546 ± 39	183	33.56
Dec. 4	2713 ± 153	717	26.44	499 ± 45	212	42.47
Dec. 11	2319 ± 182	854	36.84	452 ± 55	256	56.63
Dec. 18	2168 ± 211	991	45.72	790 ± 171	801	101.35
Dec. 26	2160 ± 186	871	40.31	362 ± 49	229	63.24
Jan. 2	1563 ± 82	383	24.48	456 ± 60	280	61.40
Jan. 8	2040 ± 172	807	39.55	490 ± 90	422	86.08
Jan. 15	1955 ± 84	394	20.13	563 ± 48	224	39.80
Jan. 22	1839 ± 160	751	40.81	704 ± 87	406	57.72
Jan. 29	2470 ± 154	720	29.15	591 ± 73	342	57.84
Feb. 5	1873 ± 144	676	36.09	546 ± 70	330	60.34
Feb. 13	2036 ± 108	505	24.80	826 ± 148	693	83.89
Feb. 19	1907 ± 146	686	35.97	893 ± 101	474	53.08
Feb. 26	2038 ± 139	651	31.96	1066 ± 100	468	43.87
Mar. 12	2007 ± 142	668	33.29	1006 ± 92	431	42.81
Mar. 19	2189 ± 129	604	27.58	857 ± 74	346	40.40
Mar. 26	2290 ± 199	934	40.76	635 ± 66	310	48.85
Apr. 2	1561 ± 185	869	55.70	856 ± 94	443	51.74
Apr. 9	2530 ± 168	787	31.09	1066 ± 98	460	43.15
Apr. 16	2765 ± 233	1092	39.50	709 ± 57	269	37.95
Apr. 23	2971 ± 200	939	31.62	916 ± 99	462	50.44
Apr. 30	2301 ± 183	857	37.23	619 ± 88	413	66.63
May 7	2574 ± 146	686	26.64	699 ± 46	216	30.95
May 14	2514 ± 150	668	26.55	622 ± 62	276	44.34
May 21	1672 ± 93	413	24.71	565 ± 36	159	28.19
May 28	2523 ± 169	752	29.78	479 ± 58	259	54.11
June 4	1960 ± 172	763	38.94	678 ± 62	276	40.77
June 11	1920 ± 153	682	35.53	509 ± 44	196	38.50
June 18	2609 ± 205	860	32.94	530 ± 42	176	33.18
Mean	2210 ± 45	363	16.40	673 ± 24	190	28.20
Minimum	1561			362		
Maximum	2971			1066		

1 and 2, illustrating the mean values, have been drawn in the form of the percentage deviations of the smoothed means from standard

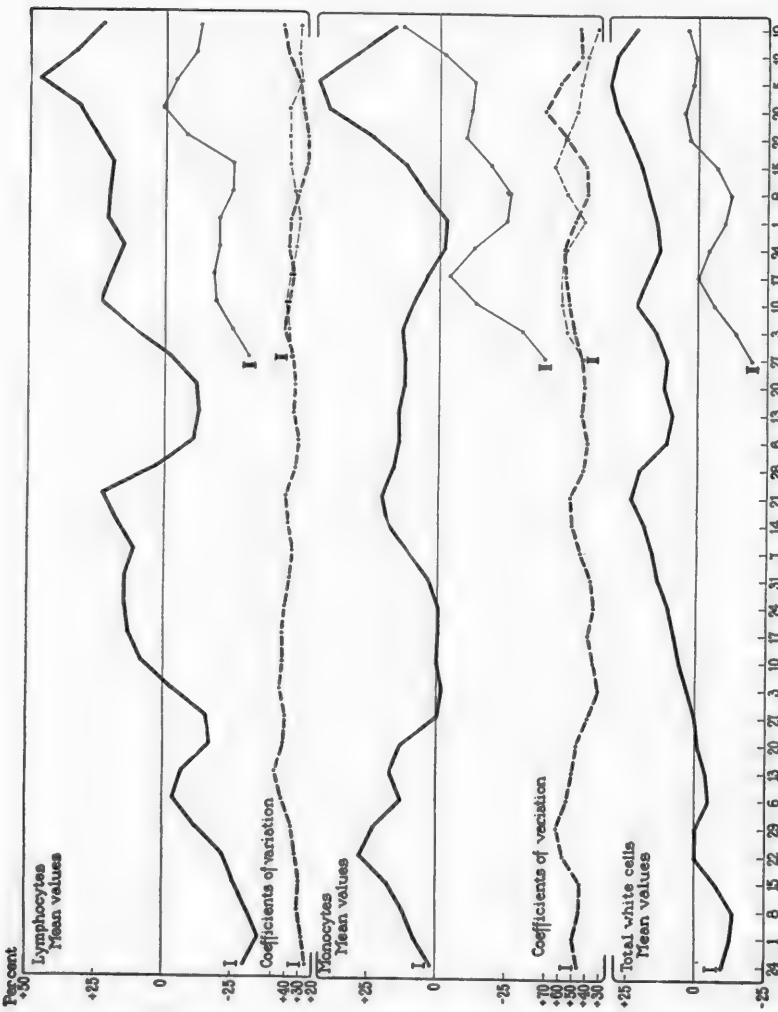
values (1, 4); similar curves for the total white cells (2) have been included to facilitate convenient comparisons with their fluctuations. The other curves in the text-figures represent the smoothed coefficients

TABLE V

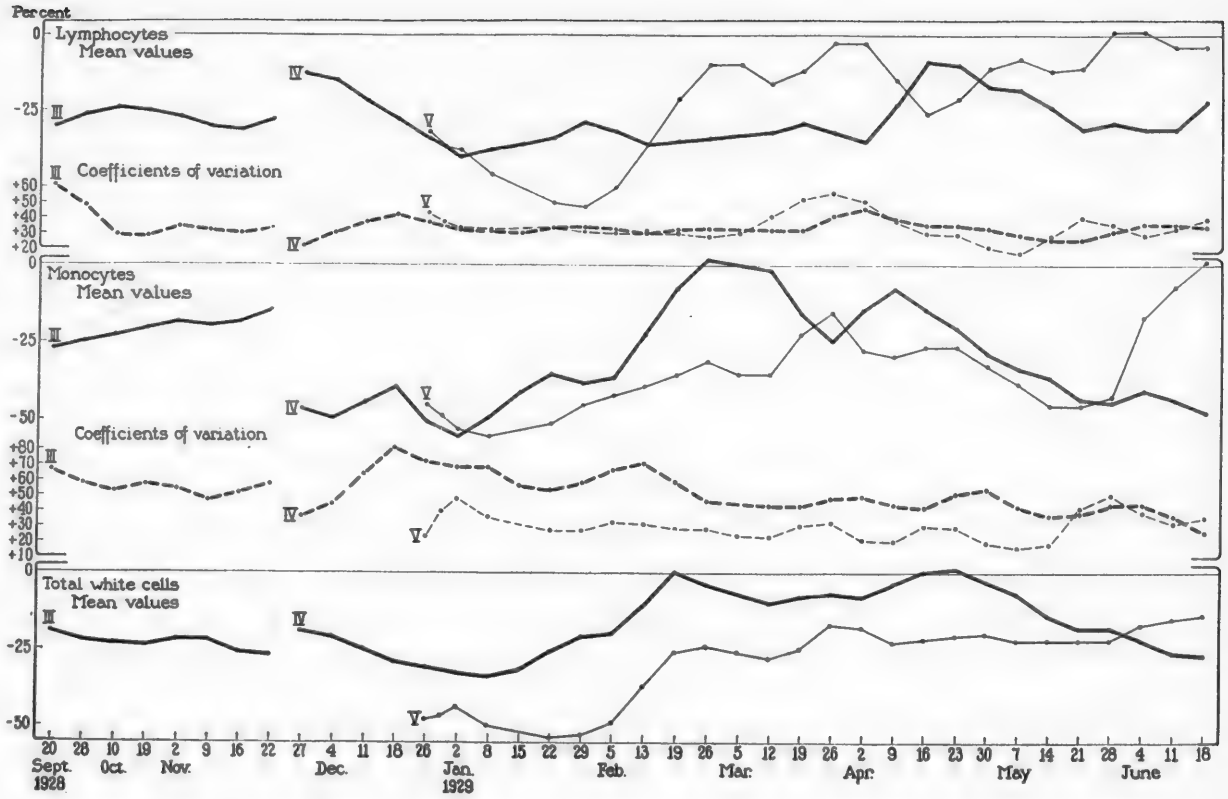
Group V—5 Rabbits. Consecutive Values for Lymphocytes and Monocytes

Date	Lymphocytes			Monocytes		
	Mean values	Standard deviation	Coefficient of variation	Mean values	Standard deviation	Coefficient of variation
1928–29	*per cmm.*	*per cmm.*	*per cent*	*per cmm.*	*per cmm.*	*per cent*
Dec. 29	2310 ± 95	314	13.59	631 ± 92	305	48.34
Dec. 31	1650 ± 206	683	41.39	350 ± 34	113	32.29
Jan. 3	2127 ± 386	1279	60.13	588 ± 65	215	36.56
Jan. 10	1676 ± 130	431	25.72	309 ± 26	86	27.83
Jan. 24	1135 ± 107	353	31.10	544 ± 60	200	36.76
Jan. 31	1503 ± 90	299	19.89	505 ± 46	153	30.30
Feb. 7	1198 ± 139	460	38.40	591 ± 44	146	24.70
Feb. 15	2147 ± 211	698	32.51	605 ± 69	227	37.52
Feb. 21	2304 ± 146	485	21.05	618 ± 47	156	25.24
Mar. 1	2941 ± 328	1087	36.96	723 ± 71	234	32.37
Mar. 8	2820 ± 156	517	18.33	639 ± 47	146	22.85
Mar. 15	2387 ± 167	552	23.13	561 ± 67	223	39.75
Mar. 22	2610 ± 214	710	27.20	816 ± 148	492	60.29
Mar. 29	3092 ± 396	1314	42.50	905 ± 133	441	48.75
Apr. 5	3038 ± 133	440	14.48	671 ± 132	436	64.98
Apr. 12	2668 ± 114	377	14.13	635 ± 54	179	28.19
Apr. 19	1990 ± 226	749	37.64	875 ± 73	242	27.66
Apr. 26	2357 ± 208	688	29.19	523 ± 60	199	38.05
May 3	2927 ± 160	529	18.07	788 ± 31	101	12.82
May 10	2700 ± 117	388	14.37	563 ± 31	101	17.94
May 17	2886 ± 139	459	15.90	524 ± 32	106	20.23
May 24	2219 ± 184	610	27.49	547 ± 85	283	51.74
May 31	3580 ± 605	2006	56.03	531 ± 56	184	34.65
June 7	2913 ± 176	584	20.05	880 ± 60	198	22.50
June 14	2913 ± 173	572	19.64	1023 ± 107	353	34.51
June 21	2944 ± 260	861	29.25	1007 ± 125	414	41.11
Mean	2424 ± 80	607	25.04	652 ± 23	176	26.99
Minimum	1135			350		
Maximum	3580			1023		

of variation of the means. The standard values used in this comparison of mean values are: lymphocytes, 3050 per cubic millimeter; monocytes, 1000 per cubic millimeter; total white blood cells, 9560 per cubic millimeter.



TEXT-FIG. 1. Mean values for consecutive lymphocyte and monocyte determinations as percentage deviations from standard values. 1927–28.



TEXT-FIG. 2. Mean values for consecutive lymphocyte and monocyte determinations as percentage deviations from standard values. 1928–29.

DISCUSSION AND SUMMARY

The results of these experiments will be discussed in chronological order. It will be noted that the observations on Group II were made during the spring of 1928 at which time Group I was still being examined, and that Groups IV and V were followed during the winter and spring months of 1928 and 1929.

Group I was examined at weekly intervals from October 24, 1927 to June 24, 1928 (Table I). From the curves in Text-fig. 1, which illustrate the lymphocyte and monocyte mean values in the form of percentage deviations from standard values as given above, it will be seen that the mean numbers of lymphocytes were considerably increased above their low initial level by the end of the experiment. This change was accomplished gradually and in a fairly consistent fashion with the exception of a minor drop of the values in December and June and an abrupt and pronounced fall at the end of February and the first half of March. In the case of the monocytes, the general level of which was continuously above the standard value, no such sustained increase of mean values was observed. The curve representing these cells contains 3 major upward swings which occurred in November, in February and March, and in May. During certain periods, as in October and November, and again in May and June, the lymphocyte and monocyte curves follow the same general trend. At other times, however, this parallel relationship did not hold. In January, the lymphocyte curve describes a sharp rise while the monocyte curve is stationary and in February, March, and April, when an abrupt fall of the lymphocyte curve is followed by a pronounced rise, the monocyte curve describes a slight but protracted decline. It will be seen by the curve in Text-fig. 1, that the total white cell count was considerably increased during the experiment, and a comparison with the lymphocyte curve shows that its fluctuations are frequently reflected in those of the total white cells. Although the coefficients of variation of the lymphocyte means (Table I) are of a high order of magnitude, the curve of the smoothed values (Text-fig. 1) is quite regular. The monocyte coefficients (Table I) are slightly higher than those of the lymphocytes and their smoothed curve (Text-fig. 1) is more irregular.

Group II was examined from March 29 to June 19, 1928 (Table II). The curves representing its lymphocyte and monocyte means (Text-fig. 1) bring out clearly the lower numerical levels of both classes of cells as compared with those of Group I. In the case of the lymphocytes the general forms of the curves of Groups I and II are very similar but with the monocytes, the resemblance is less striking.

The lymphocyte and total white cell curves are, on the whole, much alike. There are certain portions of the latter curve, however, which resemble the monocyte more than the lymphocyte curve, as for example, that covered by the last two observations. The coefficients of variations of the lymphocyte and monocyte means of Group II (Table II) are of the same order of magnitude as those of Group I and their smoothed curves (Text-fig. 1) present the same general appearance.

Group III was examined from September 20 to November 22, 1928 (Table III). From the curves in Text-fig. 2, it will be seen that the levels of both the lymphocyte and the monocyte mean values were of the same order as those of Group II rather than those of Group I. Only minor changes in the values of Group III were observed. The monocyte curve describes a gradual comparatively slight rise while the lymphocyte curve shows no clearly defined trend. The total white cell curve describes a slight downward trend and it is almost a mirror image of the monocyte curve. With the exception of the first two points of the lymphocyte curve, the curves of the smoothed coefficients of variation of the lymphocyte and monocyte means (Text-fig. 2) are comparable to those of Groups I and II.

Group IV was examined from November 27, 1928 to June 19, 1929 (Table IV). The general level of the lymphocyte mean values was comparable to the results in Groups II and III and to the first quarter of the findings of Group I. During December, the trend of mean values was decreasing as shown by the falling curve (Text-fig. 2); during January, February, and March, a low level was fairly constantly maintained; in April, an abrupt increase occurred and this was succeeded by a second decrease. The curve representing the monocytes (Text-fig. 2) shows first that the general level of mean values was usually lower than that of Group I and was more comparable to those of Groups II and III. From very low levels in December and early January, the pronounced upward swing of the curve in February, March, and April indicates the increase of monocytes during this time, and its subsequent descent illustrates the eventual drop in the numbers of these cells. The general trend of the lymphocyte and the monocyte means is similar in the beginning and during the last third

of the experiment, but during much of the time, the increase of monocytes was accompanied by practically no change in the level of lymphocyte mean values. In general form, the total white cell curve (Text-fig. 2) has many points of resemblance with the monocyte as well as with the lymphocyte curve.

The coefficients of variation of the lymphocyte means (Table IV, Text-fig. 2) which are on the whole quite uniform, are of a similar order of magnitude to those of the other groups. The coefficients of the monocytes (Table IV, Text-fig. 2) are somewhat more variable and tend to be slightly higher than those of the other groups.

Group V was examined from December 29, 1928 to June 21, 1929 (Table V). The mean lymphocyte values as shown by the curve in Text-fig. 2 were on the whole higher than those of Group IV examined during the same months, but they did not equal the high levels of Group I examined the previous year. In its first portion, the curve representing these findings resembles that of Group IV, but its rise was initiated in February and from then onward to the end of the experiment, a comparatively high level prevailed. The rise of the Group IV curve, which was of short duration, occurred in March and April. In respect to the maintenance of higher values in the spring months, Group V resembled Groups I and II. The monocyte curves of Groups IV and V are, on the whole, similar in general form until the end of the experiment (Text-fig. 2); during June, the curve for Group V describes an abrupt and marked rise while that of Group IV continues its downward trend. The general level of the Group V mean values was slightly lower than those of Group IV. It will be noted by comparing their respective curves, that the movements of the lymphocyte and monocyte mean values are generally similar in direction during the first two-thirds of the experiment. In April and May, however, the lymphocyte curve is rising while the monocyte curve is falling; in June the lymphocyte curve is stationary at a high level while the monocyte curve shows an abrupt rise. On the whole, the general contour of the total white cell curve of Group V (Text-fig. 2) resembles the lymphocyte more than the monocyte curve. During April and May, however, when its level continues to be quite regularly maintained, the lymphocytes are rising and the monocytes

are falling; in June, when it is undergoing a gradual and comparatively slight rise, the lymphocytes are high and the monocytes are very high.

The coefficients of variation of the lymphocyte means of Group V (Table V, Text-fig. 2) are entirely comparable to those of Group IV; those of the monocyte means (Table V, Text-fig. 2) on the other hand, are considerably smaller and are generally more uniform. As far as general level is concerned, the monocyte coefficients of Group V are the lowest of any of the 5 groups.

There are certain features of these results which merit special attention. In the first place, the general levels of both lymphocyte and monocyte mean values during 1927–28 were on the whole higher than in 1928–29, as was found with the red cells and hemoglobin (1), the total white cells (2), and the neutrophiles, the basophiles, and the eosinophiles (3). Secondly, the parallelism shown by two groups examined during the same months with respect to the direction of a change in the level of mean cell values and the time of its occurrence, which was characteristic of the other cells and of the hemoglobin content, was less pronounced in the case of the lymphocytes and monocytes. With Groups I and II, the lymphocyte curves are very similar but those of the monocytes are less alike; with Groups IV and V, the monocyte curves up to their last periods have a general resemblance to each other while the lymphocyte curves are frequently dissimilar. Thirdly, the general trend of cell level shown by the lymphocytes was one of increasing values in the late winter and spring months while lower values prevailed in the fall and winter. In the case of the monocytes, a similar but less consistent trend toward higher numerical values was observed and in addition to a late winter and spring rise, there was some indication that higher counts were characteristic of the early winter months (Group I, November; Group III, rise of curve from a lower September to a higher November level; Group IV, fall of curve from a higher November to a lower December level). Fourthly, the period of greatest irregularity in the lymphocyte mean values occurred in the late winter and spring months of both years; with the monocytes, on the other hand, this feature was observed in the fall and early summer months of the first year and during the late winter, the spring and the early summer months of the second year.

The significance to be attached to the major changes in the levels of lymphocyte and monocyte mean values is indicated by the following examples of the ratios of the differences of various means to their probable errors.

Group number	Lymphocytes			Monocytes		
	Dates of mean values		Ratio	Dates of mean values		Ratio
I	Oct. 24	June 19	3.26	Oct. 24	June 5	2.94
	Feb. 21	May 20	4.03	May 1	June 5	3.16
	Mar. 20	June 5	7.72			
II	Mar. 29	May 22	5.26	Mar. 29	June 19	7.91
III	Sept. 20	Oct. 19	1.74	Sept. 20	Nov. 22	0.92
IV	Nov. 27	Jan. 2	6.61	Dec. 26	Feb. 26	6.34
	Jan. 2	Apr. 23	3.45	Feb. 26	June 18	4.96
	Apr. 23	June 11	2.46	Feb. 26	Mar. 26	3.59
V	Dec. 29	June 14	3.06	Dec. 26	June 21	2.43
	Jan. 24	Mar. 8	8.92	May 17	June 14	4.46
	Apr. 19	June 7	3.23			

The question of the numerical relationship of lymphocytes and monocytes in the peripheral blood has received much attention. In so far as the results on these groups of normal rabbits are concerned, the present findings indicate that a constant and consistent relationship between these cells does not obtain over long periods of time. This feature of the observations may be best appreciated by comparing the respective curves representing the consecutive weekly mean values of these cells (Text-figs. 1 and 2). In certain portions, both curves show similar changes in level during the same weeks, indicating that both classes of cells have undergone analogous numerical changes (Group I, November, May, June; Group IV, January, April, May; Group V, February, April). In other portions, however, the curves move in opposite directions (Group I, December, January, April; Group II, June; Group III, October; Group IV, December, February; Group V, May, June). It is evident, however, that before final conclusions on the lymphocyte-monocyte numerical relationship can

be drawn, the results of experiments of this type must be analyzed from the standpoint of the individual animal.

CONCLUSIONS

Consecutive weekly observations on the lymphocyte and monocyte counts of the peripheral blood were made on 5 groups of normal rabbits, a total of 45 animals, during a period of 20 months from October, 1927 to July, 1929. Individual groups were examined 8 to 35 weeks. The results are analyzed on the basis of the mean group values of each week.

In the case of the 4 groups followed 13 to 35 weeks, there was a general tendency for the lymphocyte mean values to become increased; with the group observed 8 weeks, the level of mean values showed little change. The general trend of the monocyte mean values was also in the direction of higher levels but it was less pronounced than that of the lymphocytes.

The period of greatest irregularity in the mean values of the lymphocytes was in the late winter and spring months of both years. With the monocytes, periods of fluctuating values occurred in the fall of 1927, the spring and early summer of 1928, and in the late winter, spring, and early summer of 1929.

There was a certain degree of parallelism in the case of two groups examined during the same months with respect to the direction and time of occurrence of a change in the level of lymphocyte and monocyte mean values.

The general levels of lymphocyte and monocyte mean values in the groups examined during 1927–28 were higher than in the groups of 1928–29.

The results based upon the trends of mean group values obtained from consecutive weekly observations showed no evidence of a consistent numerical relationship between lymphocytes and monocytes.

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EXPERIMENTAL NEPHRITIS IN THE FROG

I. The Anatomical Evidence of Damage*

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Plates 7 to 9

(Received for publication, April 25, 1930)

In the complex problems which have arisen in the study of nephritis the experimental method has proved a useful adjunct. Many aspects of the disease which can be examined only at the end of long continued processes in man and which, therefore, remain obsure to purely anatomical investigation, become relatively simple when examined during their development by this method which allows freedom of procedure and more rigid control to the investigator.

In one aspect, however, the advance has not been encouraging, and that is the correlation of disturbances in the function of the organ with changes in its anatomical structure. Various reasons are evident for this situation. The normal function of the kidney is far from understood, and this is particularly true of the complex kidney of mammals. A further complicating factor is that the nephritis which develops under experimental conditions is of almost as involved a nature as that found in the disease of man.[1] The first and great advantage of the experi-

* This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacology and Chemistry, American Medical Association.

[1] An example of this is found in the different interpretations which have been offered for the histiogenesis of experimental chronic uranium nephritis in mammals. Dickson (*Arch. Int. Med.*, 1909, **3**, 375; 1912, **9**, 557) believes the chronicity of the lesion to be the result of a vascular lesion; Susuki and Aschoff (*Verhandl. Deut. path. Gesel.*, 1912, **15**, 199) to a hydronephrotic factor, and Oliver (*Journ. Exp. Med.*, 1915, **21**, 425; 1916, **23**, 301) to a direct inflammatory stimulation of the connective tissue.

mental method, namely, the simplification and control of conditions, is not therefore really available in its full extent when mammals are subjected to its procedures.

An analogous situation has been recognized by the physiologists in their work and these difficulties met by modification of the direction of their attack. The work of Richards (1), Bieter and Hirschfelder (2), Marshall (3), and Höber (4), to mention but a few, in which direct methods have been applied to simple kidneys has objectively established a few certain facts and thus removed them from the field of hypothesis where they had so long remained. The application of these newer methods to the study of the abnormal kidney is an obvious step. Direct experimentation, as Richards has termed it, both by visual observation of the living functioning organ and by perfusion of it, a method so brilliantly used by Höber and his pupils, might be used with the simple kidney of the frog in which abnormalities have been produced. It is possible that this might allow of the establishment of a base from which a later extension to the more complicated conditions obtaining in the abnormal mammalian kidney could be taken.

The present investigation is the first of a series of such studies of experimental nephritis in the frog.[2] As a first step, the method of production of the experimental lesions in the frog's kidney is described and the types of nephritis which may be produced in the animal detailed. In subsequent articles changes in the circulatory reactions of kidneys in which an experimental nephritis has been produced will be described and contrasted with those of the normal kidney. The response of the kidney, as tested by the method of perfusion, to damage of limited extent and of controlled nature as it may be produced in the isolated organ will next be given. The final step will be the presentation of a similar functional study of the disease as it exists when produced in the living frog and a correlation of these changes with the anatomical findings in the light of what we have learned with this "artificial" nephritis in the isolated organ.

[2] Four studies preliminary to this series, concerned with certain physiological aspects of our problem have appeared in the *Journal of Experimental Medicine*, 1929, **50**, 15; 1929, **50**, 601; 1930, **51**, 161; and the *American Journal of Physiology*, 1930, **93**, 363.

Methods

The animals used were chiefly *R. catesbiana*, since their kidneys are large and show the lesions most satisfactorily.

In our choice of renal poisons we have been guided by past studies of the similar problem in mammals. The list of agents we have used are: corrosive sublimate, potassium bichromate, uranium nitrate, ricin, cantharidin and snake venom. The first three substances have been classified from the lesions which they produce in mammals as tubular poisons, while the last three are reported to affect with more or less specificity the vascular apparatus of the kidney.

The experimental procedure was as follows.

Various amounts of a substance were injected into the dorsal lymph sacs of the frogs and the animals returned to their water tanks. After definite periods of time they were killed and portions of their kidneys fixed in 10 per cent formalin, Orth's fluid, Bensely's mixture, and Kolster's fixative. Sections were stained with hematoxylin and eosin, Van Gieson's mixture, iron hematoxylin, Mallory's connective tissue stain, Weigert's fibrin stain and for the mitochondria by Bensely's and Kolster's methods. In all cases great care was taken to fix the kidney tissue promptly after death. Post mortem change is extremely rapid if the frog lies dead in the tank and the autolytic changes resemble somewhat those which may occur as a result of toxic substances. For this reason frogs found in the morning, which might possibly have died earlier in the night were discarded and dying animals were killed as soon as their condition was detected. An apparent variation was frequently noted in the toxicity of a given substance when administered to different frogs. This we think was the result of diffusion of the substance from the dorsal lymph sac of the frog through the puncture wound into the water of the tank. Such irregularities may be largely avoided by intraperitoneal injection but the possibility of their occurrence must be kept in mind in the comparison of lesions in different animals.

We would call attention to the fact that the frog may suffer from spontaneous lesions of the kidney. These are the result of a general parasitic infection of the animal in which focal lesions develop in the kidney as well as in other organs. These lesions, consisting of circumscribed round areas of necrosis filled with nuclear débris which involve all the tissue cells indiscriminately in the infected focus, may be easily recognized. All frogs which were found to be so infected were discarded.

EXPERIMENTAL

The Lesion with "Vascular" Poisons

We were unable to produce constant lesions in the kidneys of the frogs which had been injected with the so-called vascular poisons. In the case of cantharidin the insolubility of the substance in water requires a solvent, acetic ether, which is even more toxic than the drug. The animals succumbed in a short time to the injection 0.5 cc. and if given in lesser amounts so little cantharidin was administered that no ill effects were noted after repeated injections. Large repeated injections of ricin, totaling 10 cc. of a saturated saline solution in the course of 3 days, produced no ill effects and the kidneys appeared entirely normal. The animals were also resistant to the action of snake venom. Doses which would be enormous for mammals did not kill the frogs, and when death followed repeated injections the kidneys showed no definite or constant effects.

These experiments, on account of their negative findings, are not described in detail but may be summarized by the statement that we were unable to produce lesions with regularity by the use of these substances.

The Lesion with "Tubular" Poisons

Corrosive Sublimate

The amount of corrosive sublimate injected into the animal varied from 1 to 7.5 mg. The average weight of the frogs being 500 gm. this equals an average dosage of 8 mg. per kilo. As mentioned before considerable variation in the apparent effect of the drug was noted but these irregularities were avoided by disregarding those animals in which the experiment had failed to damage the kidneys.

As in mammals a striking feature of the action of the sublimate was the damage to the tubular epithelium (Fig. 1).

This was apparent within 18 hours following the injection and in this early period consisted of marked cloudy swelling of the tubular epithelium with occasional necrosis and desquamation. The seat of the lesion was definitely limited to

the broad proximal convoluted segment of the renal tubule, the narrow neck, distal convoluted portion and the collecting tubules remaining essentially normal.[3]

In the mitochondrial preparations the usual evidences of early cellular damage were found. The granules in the involved portion of the tubule were swollen and irregularly distributed in agglutinated clumps and masses. In some necrotic cells these irregular clumps of heavily stained material persisted while in others solution of the substance had occurred so that the dead cell body was free of any granular substance. In these early stages of the damage the batonnets of the distal convoluted tubule stained normally.

In striking contrast to the effects of sublimate on mammals was the early and constant evidences of damage which were observed in the glomeruli.

In many cases these lesions were present in kidneys whose tubules showed little if any abnormalities and they were constantly found in those where the damage was at all severe. The simplest abnormality observed was the deposit of granular material in Bowman's space. This material was in some cases arranged in fibrin-like threads but its staining reaction was not that of typical fibrin. In other cases this material was condensed and deposited on the surface of the glomerular tuft where it formed a thin dense covering which followed the indentations between the capillaries into the central portions of the structure. Such appearances were, however, usually noted in kidneys which were more severely damaged than those which we are at present describing. Beside these lesions in the tubules and glomeruli the interstitial tissue showed a slight edema, and some general hyperemia of the capillaries between the tubules was occasionally present.

In animals which had received the larger doses and which had lived 70 hours the lesions were much further developed and consequently were more severe.

Extensive necrosis of the tubular epithelium was present and the desquamated dead cells filled the lumen of the proximal convoluted tubule. This portion in fact no longer existed except as a space within the membrana propria which was packed solid with granular débris. The damage in such kidneys had also spread both back toward the glomerulus and in the opposite direction, so that examples might be found where the neck and the distal convoluted tubule were also destroyed. The collecting tubules were only slightly altered. In them, however, was found débris, evidently derived from higher reaches of the tubule, and here

[3] We have used the terminology of Stewart (5) because of its analogies with that used for the mammalian kidney. Proximal convoluted tubule is synonymous with Segment II, distal tubule with Segment III, and collecting tubule with Segment IV of other writers.

also definitely formed casts were present. They were of the hyaline type as a rule, though some contained granular material. It was a striking finding that such fully formed consolidated casts were found almost only in the distal convoluted tubule and in the excretory ducts and were seldom seen in the proximal portion of the tubule where the active damage was occurring. For this reason in a frontal section through the kidney a peculiar distribution of them was found since they were clustered in those sections of the tubule that lay around the glomeruli in the middle and ventral portions of the kidney.

The glomeruli of such kidneys showed even greater damage than has been described. The same collections of fibrinoid substance in Bowman's space were found but in the tissues of the tuft definite changes were also observed. Intense engorgement with the formation of hyaline thrombi composed of agglutinated red blood cells and scattered areas of edema and even necrosis were present Patches of nuclear débris were scattered among the loops and occasionally a tuft could be found so severely damaged that hemorrhage had occurred into the capsular space. On the other hand some damaged glomeruli were anemic, and might be found greatly altered and even disrupted without any escape of blood from their capillaries. These glomerular lesions were at times the most striking abnormality found in the kidneys. In the kidney of one animal which had received 1 mg. and was killed on the second day the tubules showed no very definite lesions, yet the glomeruli were severely involved.

In the tubules of the kidneys of animals which showed these severe glomerular lesions beside the hyaline and granular cast mentioned above, red blood cells were occasionally found either still discrete and in varying degrees of disintegration or consolidated into definite blood casts (Fig. 2).

Beginning about 3 or 4 days after the injection of the toxic substance reparative processes could be found in the kidneys.

These consisted of regenerative proliferation of the epithelium of the tubules which had been damaged. In the persisting cells, scattered through the region of the tubule which was most severely involved, and at the junction of this part with portions of the tubule which were relatively undamaged, mitotic figures were frequent. One such source of new formed cells was regularly found in the collecting tubules which were rarely damaged by the toxic agent. Cross sections of these tubules showing marked proliferative changes in the epithelium might be found scattered among the necrotic tubules of kidneys which had been so severely damaged as to show destruction of not only the proximal convoluted tubule but of the distal as well.

The new formed cells could be recognized by their unusual appearance. Their nuclei were large, often twice the size of those of the original tubule cell and were as a rule oval in shape. Their chromatin was increased in amount and stained quite heavily. Such nuclei were both excessive in number and irregularly dis-

tributed so that they formed irregular giant cell-like masses covered with protoplasm which projected irregularly into the lumen of the tubule. From such centers of proliferation the cells spread out over the denuded membrana propria. These new formed cells were found apparently creeping beneath detritis and casts which filled the tubule lumen so that in specimens 14 days old the lining of the tubules was found to be completely restituted, though the new cells could be still distinguished from the regularly arranged lining of the original epithelium.

Repair in the glomeruli seems to be a much slower process. The remains of the early lesions could still be made out after 14 days, a time when the tubular damage had been largely repaired. The more acute process of hyperemia had disappeared from the tuft but granular material and fibrinoid substance was still present in Bowman's space. Very occasionally mitotic figures were found in the tissues of the tuft, but no definite "chronic" lesions such as organization or definite proliferation of the tuft tissues were present.

Potassium Bichromate

The lesions after potassium bichromate resembled in a general way those following the administration of corrosive sublimate. Certain minor differences were observed, however, particularly in the early phases of the damage or in kidneys that had only been slightly affected. The amount of drug given varied from 0.5 to 5 mg. per animal so that the dosage varied around an average of 5 mg. per kilo.

In the kidneys of frogs which were killed within 24 hours after smaller doses or in animals which succumbed in 2 or 3 days but whose kidneys were but moderately damaged, only the earlier and milder types of degeneration were found in the tubular epithelium. The lumen of the tubule throughout its length was filled with granular detritis, the cells showed a cloudy swelling with the presence of an occasional pycnotic nucleus, but little evidence of frank necrosis was present. This cellular damage, as in the case of corrosive sublimate, was limited to the proximal convoluted tubule.

In kidneys showing such slight lesions, the glomeruli, however, were almost constantly involved. Granular or fibrinoid material in Bowman's space with desquamation of the capsular epithelium, hyaline thrombosis and occasional small areas of necrosis with hemorrhage into the capsular space could be found (Fig. 3). In the interstitial tissue hyperemia was often intense, the capillaries between the tubules being crowded with red blood cells. Only a moderate number of casts of the hyaline type were present in the distal convoluted tubule and in the collecting tubules, though granular material was present throughout the entire renal unit. Occasionally collections of red blood cells still discrete or partially disintegrated could be found in the tubule lumen or were definitely formed into blood casts.

In more severely damaged kidneys the microscopic picture resembled even

more closely that seen after corrosive sublimate, for tubular damage was now equally severe, the entire proximal convoluted portion being necrotic and the tubule transformed into a mass of dead desquamated cells. This damage also spread both into the narrow neck and into the distal convolution. The glomerular lesions, exudation of granular and fibrinoid material, necrosis and hemorrhage, were more pronounced and casts of all sorts were present in the lumina of the tubules (Fig. 4).

Beginning at the third or fourth day regenerative changes in the damaged tubules were apparent. Their course was similar to that seen after sublimate, large irregular masses of epithelium filling the tubule lumen with giant cell-like structures. Mitotic figures were frequent among their large oval nuclei (Fig. 5). Repair was only slight in the glomeruli and in most cases proliferative changes could not be definitely determined.

The lesions after bichromate poisoning therefore differ somewhat from those following the administration of sublimate in that although the glomerular damage was equally intense the tubular lesions were somewhat slower to develop. In the end, however, they became equally severe.

Uranium Nitrate

After the administration of from 5 to 10 mg. of the uranium nitrate, averaging 15 mg. per kilo, lesions developed resembling those following potassium bichromate more closely than those produced by corrosive sublimate. On account of this similarity they will be only briefly described.

In cases of mild damage or during the first 24 hours of the development of the lesion, the tubules showed only slight evidences of cellular damage such as cloudy swelling and occasional necrosis. The glomeruli were regularly involved by the milder changes which produced an accumulation of protein material in the capsular space (Fig. 6). As the poisoning became more severe or the slighter changes more fully developed, the tubular damage was more pronounced until it equalled that seen with potassium bichromate (Fig. 7). It was only occasionally, however, that the typical picture following corrosive sublimate could be obtained, that is, a complete destruction of the entire proximal convoluted tubule and the replacement of it by masses of dead disintegrating cells. The interstitial lesions of edema and hyperemia, though present, were also less pronounced.

In the severely damaged kidney, the appearance is approximately the common picture seen after all these toxic agents. The glomeruli were engorged, hyaline thrombi were present, small areas of necrosis of the tuft could be seen and hemorrhage into Bowman's space (Fig. 8). In the proximal convoluted portion definite

necrosis with desquamation filled the lumen of the tubule with débris, and casts, both granular and hyaline, were present in the distal convoluted and and collecting tubules.

In animals which lived from 4 days to a week the irregular regenerative proliferation of the surviving epithelium in the damaged tubule was very apparent. In one animal which was killed 18 days after the administration of the poison the necrotic material and other evidences of early damage were almost entirely removed, only an occasional cast still persisting in the tubules, yet the abnormal condition of the proximal convoluted tubule was apparent in the excessive number of atypically new formed cells which formed its lining.

DISCUSSION

In a general way the lesions produced in the frog's kidney by potassium bichromate, corrosive sublimate and uranium nitrate are similar and resemble those which have been described as occurring in mammals. The significance of the differences observed, however, requires a brief discussion.

Of the tubular apparatus, in the frog as in the mammal, it is the proximal convoluted tubule which suffers most severely. For the latter group this point was first clearly established by Susuki (6) in his studies by means of vital staining. Although such special methods are required for the recognition of this point in the complex mammalian kidney, in the more simply arranged mesonephros of the frog the localization of the damage in the broad proximal convoluted tubule can be easily determined by means of its characteristic morphology and by the situation of this segment in the organ. As in the mammalian kidney, so too in the frog's mesonephros this limitation of the damage to a restricted portion of the tubule is observed only with small or moderate doses of the toxic agent. After the administration of large doses the damage spreads both toward the glomerulus and into the distal convoluted tubule. The straight portion of tubule connecting the two convoluted portions is thus involved as well in the lesion and though it can hardly be considered a loop of Henle, nevertheless its involvement in the spreading lesions is analogous to the fate of that structure under similar conditions in the mammal.

It is corrosive sublimate in the frog as in the mammal which produces the most extreme picture of tubular damage. We might point out, however, that the striking appearances seen after this substance

do not necessarily indicate a lesion of any greater severity than that which follows potassium bichromate or uranium nitrate where the histological picture is less impressive. In both cases the cells are killed but after sublimate the coagulation of the débris into massive blocks of deeply staining material contrasts with the disintegration of the dead cells which occurs after the administration of the other two poisons. The possibility of error in the estimation of the quantitative relation of the damage in the different forms of poisoning is therefore obvious.

In the effect of these "tubular" poisons on the glomeruli of the frog's kidney differences which at first hand may seem to be significant are observed. After all the poisons the glomeruli were regularly and definitely damaged. This damage was of equal degree to that seen in the tubules; in fact observations were recorded where the glomerular lesion was plainly evident in kidneys whose tubules showed very slight if any morphological evidence of involvement. The exudation of protein into Bowman's space in both granular and fibrinoid form, necrosis and thrombosis in the tuft tissues and hemorrhage from ruptured capillaries were regularly present. Such a regularity of striking morphological lesions has not been found in the mammalian kidney after similar treatment. It is a fact of great importance, nevertheless, that such lesions do occur though as a rule they are inconstant in occurrence or so slightly developed as to require some attention for their observation. After corrosive sublimate, both in animals and in fatal poisoning in man, it is not unusual to find protein material exuded into Bowman's space, and there is other evidence that the vessels throughout the kidney have also been damaged. Oertel (7) has emphasized the importance of these changes. Glomerular lesions of slight degree such as pycnosis of the nuclei of the tuft cells and the occurrence in them of hyaline degeneration have been described by Christian and O'Hare (8) as occurring occasionally after uranium poisoning. Furthermore the experiments of Wiesel and Hess (9) have shown that those glomeruli which may appear normal to morphological examination after the administration of uranium nitrate are in fact severely damaged, for a subsequent injection of adrenalin causes their weakened capillaries to burst and produces glomerular hemorrhages throughout the kidney.

It would seem, therefore, that the glomerular lesions which in mammals present only slight or inconstant morphological evidences of damage, are regularly observed, well developed and visible to simple methods of demonstration in the frog's kidney. The differences between the lesions in the two classes of animals is only a quantitative one, for qualitatively they are essentially similar.

The frog's mesonephros is particularly well suited therefore for the study of lesions which remain masked in the complex kidney of mammals. The importance of this point which forms the basis of our entire program of investigation will become increasingly apparent as the results of our investigations of nephritis in the frog by other methods are given. For this reason it is obviously too early a point in our work to discuss either the "nature" of the changes which are observed in these forms of kidney damage, or to attempt a classification of the toxic substances as either "tubular" or "vascular" since but one aspect of the problem, the morphological, has been given. The equally important functional manifestations of the lesions will be next considered.

SUMMARY

1. The damage produced by corrosive sublimate, potassium bichromate and uranium nitrate in the frog's kidney is described.

2. The morphological lesions consist of evidences of tubular damage, such as regressive changes in the epithelium, and of damage to the glomeruli ranging from increase in their permeability to gross damage of and hemorrhage from the tuft.

3. The point is emphasized that these lesions differ in their degree rather than in their nature from those found in the mammalian kidney after the administration of the same poisons.

4. The frog's kidney is exceptionally well suited therefore for the study of lesions which though present are masked in the complexities of structure and function of the mammalian kidney.

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EXPLANATION OF PLATES

Unless otherwise stated the sections of kidney were stained with Delafield's hematoxylin and eosine.

PLATE 7

FIG. 1. Kidney from an animal killed 48 hours after the injection of 5 mg. of corrosive sublimate. The section shows the central and dorsal zones (Stewart) of the kidney, the glomeruli and distal convoluted tubules lying in the former (upper half of section) and the broad proximal convoluted portions in the latter (lower portion of section). There is some granular material in the capsular spaces of the glomeruli. The cells of the proximal segment of the tubule are mostly necrotic and desquamated. Clustered around the glomeruli, the distal convoluted portions still show nuclear staining as do many of the straight portions which connect the two convoluted segments. 130×.

FIG. 7. Section shows the junction of the central and dorsal zones of the kidney of a frog which was killed 72 hours after the injection of 5 mg. of uranium nitrate. The capsular spaces of the glomeruli are filled with granular débris. There is widespread necrosis and desquamation of the epithelium of the proximal convoluted tubule in the lower part of the section, and somewhat less pronounced but similar lesions in the distal portion above. 130×.

PLATE 8

FIG. 2. The central zone of a kidney from an animal killed 72 hours after the injection of 1 mg. of corrosive sublimate. The section shows the glomeruli and surrounding distal convoluted segments. Although the epithelium of these portions is fairly well preserved many casts composed of disintegrated and agglomerated red blood cells are seen in their lumina. 120×.

FIG. 3. The junction of the central and ventral zones of the kidney of a frog killed 48 hours after the injection of 5 mg. of potassium bichromate. Lighter areas of necrosis containing nuclear débris are seen in the greatly swollen glomeruli. In spite of this glomerular damage the sections of tubules in the lower left corner, which are the terminal portions of the proximal convoluted tubule, show no definite lesions. 140×.

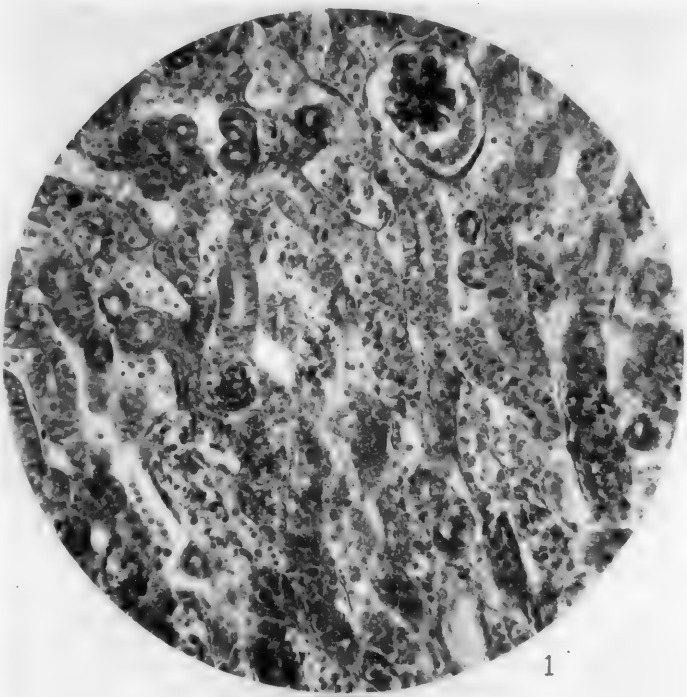
FIG. 4. Kidney of a frog killed 72 hours after the injection of 2 mg. of potassium bichromate. The section is stained with Mallory's connective tissue stain. Only a portion of the central zone of the kidney is shown. In this zone the distal convoluted tubules are filled with hyaline casts (deep blue). Covering the glomerular tuft and extending into it is a layer of fibrinoid material, which also stains deep blue rather than red as would true fibrin. 170×.

PLATE 9

FIG. 5. Section through the dorsal zone of a kidney of a frog which had been killed 120 hours after the injection of 5 mg. of potassium bichromate. Stained with iron hematoxylin after formalin fixation, a method which does not preserve the granular elements of epithelium. The lining of the proximal convoluted tubule, the only segment shown in the section, is composed of an excessive number of large regenerated cells whose nuclei are oval, and irregular in size, shape and distribution. A mitotic figure is seen in one of the upper cross sections. 500×.

FIG. 6. Section passing through the junction of the central and dorsal zones of a kidney from an animal which had been killed 72 hours after the administration of 5 mg. of uranium nitrate. The capsular space of all the glomeruli is filled with granular exudate. The cells of the distal convoluted tubules show moderate damage, but much less than the proximal convoluted segments which are not shown in the figure. 140×.

FIG. 8. Section shows a small portion of the central zone of a kidney from a frog killed 24 hours after the injection of 8 mg. of uranium nitrate. The sections through the distal convoluted tubules show them to be apparently normal. In the glomerular space is a recent hemorrhage from the tuft. The nucleated red blood cells are still well preserved. 300×.



(Oliver and Smith: Experimental nephritis in the frog. I)

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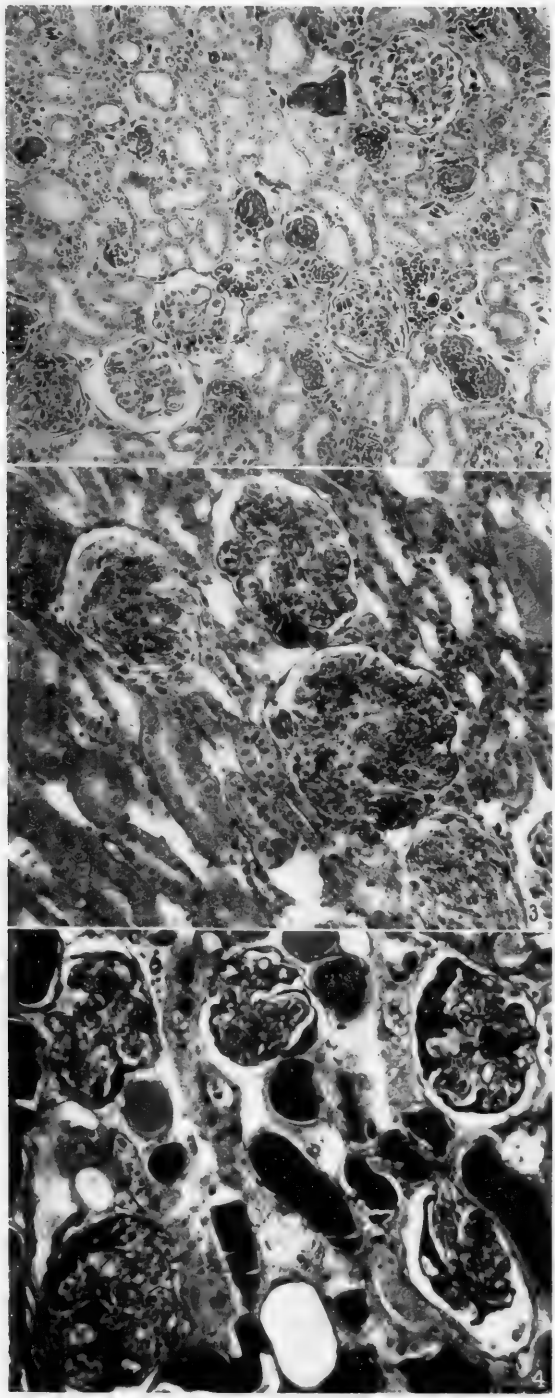

(Oliver and Smith: Experimental nephritis in the frog. I)

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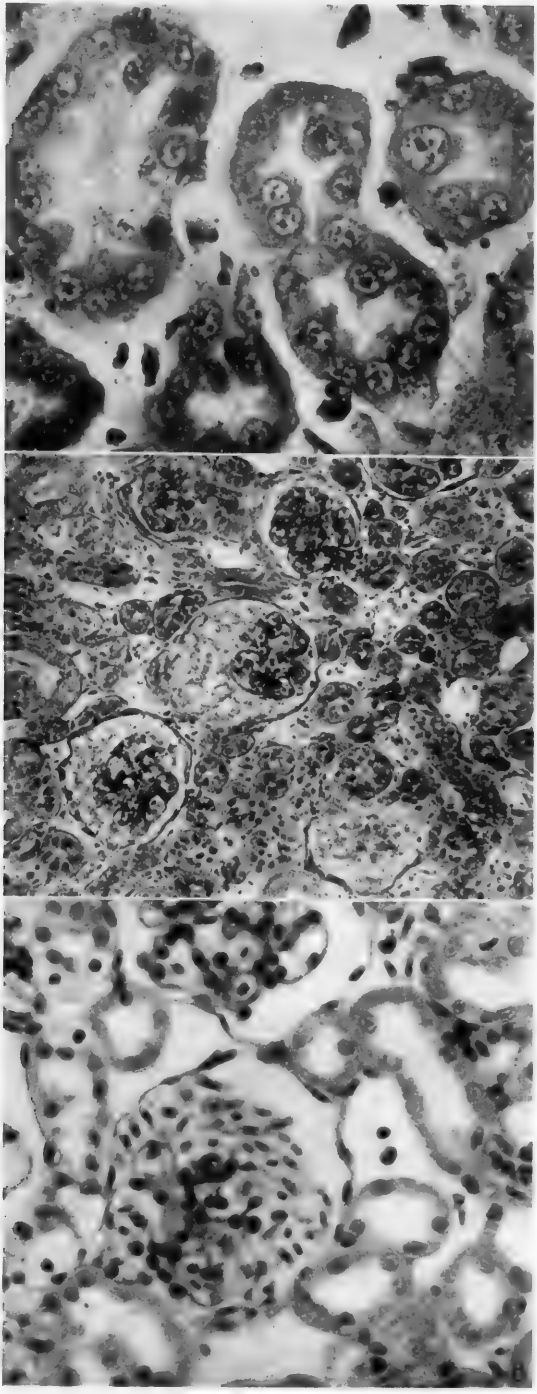

(Oliver and Smith: Experimental nephritis in the frog. I)

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A STUDY OF THE RELATIONSHIP OF THE SCROTAL SWELLING AND RICKETTSIA BODIES TO MEXICAN TYPHUS FEVER

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(Received for publication, April 30, 1930)

In the study of typhus fever at the present time, etiological investigation turns to a considerable extent upon whether or not the small rickettsia-like bodies described in the scrotal swellings by Mooser can be looked upon as the causative agents, or whether they represent merely a secondary infection carried along through generations of guinea pigs together with the typhus virus.

Some light may be thrown upon this question by the occasional strains of Mexican typhus fever in which scrotal swelling does not occur for several guinea pig generations and then, for unknown reasons, reappears. Such strains have been noted from time to time by ourselves as well as others, and the purpose of the present paper is to describe the results of guinea pig inoculations in which a number of strains taken directly from patients were carried on in series of considerable length with irregularity in the appearance of swelling. The experiments described deal with a strain isolated at Jilotzingo which is spoken of briefly as the "J" strain.

Appearance of Scrotal Swelling in Guinea Pigs Directly Inoculated from Patients

Table I shows that inoculations from three patients in the same epidemic, the blood of each injected into three guinea pigs, resulted in positive reactions in all three of the guinea pigs from Patient 1; in none of those inoculated from Patient 2 and in only one guinea pig inoculated from Patient 3. In only one animal of the four successful inoculations—successful as far as fever reaction is concerned—did scrotal swelling appear, but it is important to note that the typical

swelling in this case was produced directly as the result of inoculation with human blood.

The Frequency of Scrotal Swelling in Guinea Pigs Inoculated in Series with the "J" Strain

The observations recorded in Table II are important in that there was only an occasional scrotal reaction in the guinea pigs inoculated up to the sixth generation. Then, for eight generations, from the seventh to the fifteenth, the scrotal reaction was absent. After that it recurred in two guinea pigs of the sixteenth generation. It was

TABLE I

Patient	Guinea pig	Results
1	1	Fever, no swelling
	2	Fever, swelling
	3	Fever, no swelling
2	4	No fever or swelling
	5	No fever or swelling
	6	No fever or swelling
3	7	No fever or swelling
	8*	Fever
	9	No fever or swelling

* Guinea pig 8 was a female, the others being males.

In this experiment guinea pigs were carefully selected to avoid contamination with Salmonella, which frequently occurs in Mexico City.

lost again from the seventeenth to the twentieth generation, and again from the twenty-second to the twenty-third, but reappeared in the twenty-fourth generation.

Rickettsia were not found in any of the animals showing swelling until the twenty-fourth generation. This we are inclined to attribute to the fact that we had not yet learned that the rickettsia are difficult to find unless the examination is made quite early. At the height of the swelling—4 to 6 days after inoculation—the rickettsia are much more difficult to find than when the tunica is examined at the very first indication of reaction, or even before reaction has become manifest.

At any rate, the experiments recorded in the table show that after prolonged failure of scrotal swelling, the typical Neill-Mosser reaction with rickettsia can appear, a point that would suggest strongly that the rickettsia and the lesion in the tunica vaginalis are truly part of the typhus fever and not incidentally carried along.

Cross Immunity Experiments

We considered it of some importance also to determine whether a typhus fever without scrotal swelling in guinea pigs immunized to subsequent inoculation with a Mooser strain which produced swelling

TABLE II

No. of passage	Material injected	No. of guinea pigs with typhus fever	No. of guinea pigs with swelling	Rickettsia	No. of guinea pigs with no swelling
1st	Human blood	4	1	0	3
2nd	Guinea pig blood	5	0		5
3rd	Guinea pig blood	5	1	0	4
4th	Guinea pig blood	6	2	0	4
5th	Guinea pig blood	4	1	0	3
6th	Guinea pig blood	4	1 (slight)	0	3
7th to 15th	Guinea pig blood	24	0		24
16th	Guinea pig blood	3	2	0	1
17th to 20th	Guinea pig blood	10	0		10
21st	Guinea pig blood	2	1	0	1
22nd to 23rd	Guinea pig blood	4	0		4
24th	Guinea pig blood	2	1 (early castrated)	++++	1
25th	Guinea pig blood	2	0		2

with considerable regularity. Of thirty-three guinea pigs inoculated with the "J" strain in this series, only four showed scrotal swelling. Nine were unsuccessfully inoculated, showing neither fever nor swelling. Twenty, which showed typical fever curves but no swelling of the scrotum, were subsequently reinoculated with the typical Mooser strain and in no case developed either swelling or fever.

The nine unsuccessfully inoculated pigs were all reinoculated similarly and all of them developed both fever and swelling.

From these experiments it is clear that a typhus fever conveyed

by the "J" strain and recovered from without scrotal lesion protects against the scrotal lesion upon subsequent inoculation with the typical Mooser strain.

This would again indicate that the scrotal lesion is merely an incidental localization in some guinea pigs of the disease which is immunized against by a general typhus fever, even if in the course of the latter this lesion is absent.

TABLE III

Guinea pig	Passage No.	Fever	Swelling	Rickettsia
a	2nd	+	−	
b	3rd	+	+	0
c	4th to 15th	+	−	
d	16th	+	+	0
e	17th to 20th	+	−	
f	21st	+	+ slight	0
g	22nd to 24th	+	−	
h	25th	+	+	Very few extracellular

From Guinea pig "h" (25th passage) lice were then intrarectally inoculated with tunica by the Weigl method and after 10 days this material was injected into guinea pigs with the following results:

Guinea pig	Lice strain. Passage No.	Fever	Swelling	Rickettsia
i	1st	+	+	++++
j	2nd	+	+	++++
k	3rd to 5th	+	−	
l	6th	+	+	++++

The Influence upon the Ability of a Strain to Cause Scrotal Swelling after Passage through Lice

Table III again shows the "J" strain, carried in twenty-five generations with frequent absence of scrotal swelling for a number of generations, even after the twenty-fifth generation is passed through lice and reinoculated into guinea pigs. In the first two generations after passage through lice, typical swelling with abundance of rickettsia

occurred, then again for two generations swelling and rickettsia were absent, in spite of typical febrile reactions, to reappear in the sixth generation as markedly as they had been present before.

SUMMARY

The experiments recorded above have demonstrated the following points:

1. Scrotal swelling can appear in guinea pigs directly inoculated from a human case of Mexican typhus fever.

2. In certain strains of this disease, a number of generations of guinea pigs may show absolutely no scrotal swelling, which, however, may reappear in subsequent animals, suggesting—though not absolutely proving—that the scrotal swelling is an integral part of the disease and is not due to an incidental accompanying organism. If the latter were true, one would expect the organisms that caused the scrotal swelling to disappear during the negative generations.

3. A typhus fever sustained by a guinea pig without scrotal swelling protects against the swelling upon subsequent inoculation with a strain which produces this with considerable regularity.

4. Louse passage increases the capacity of a strain to produce the scrotal lesion, probably because of the considerable accumulation of rickettsia in the louse, but in the experiment noted, even after louse passage, two generations without swelling occurred, followed by reoccurrence of the swelling.

We believe that these observations, taken together, can be interpreted in favour of the likelihood that the swelling is a part of the disease and that the rickettsia-like organisms described by Mooser in the tunica vaginalis have etiological significance.

STUDIES ON INFLAMMATION

IV. Fixation of Foreign Protein at Site of Inflammation

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(Received for publication, May 14, 1930)

In the previous papers of this series it was shown that trypan blue injected directly into an inflamed area was fixed *in situ* and failed to reach the tributary lymph nodes (1). When this dye was introduced into the circulating blood stream it rapidly entered the inflamed area and was fixed there. Subsequent studies showed that the rapid accumulation of dye in an inflamed area is the result of increased capillary permeability (2). These studies were then extended and it was found that colloidal iron or ferric chloride injected directly into an inflamed area was fixed *in situ* by the inflammatory process, and that ferric chloride injected intravenously rapidly entered inflamed cutaneous areas, where its presence was identified by both qualitative and quantitative determinations (3).

The earlier literature on the dissemination of substances from the site of injection has been reviewed in previous publications (1 and 2). Opie (4) showed that foreign protein injected into the skin of an actively immunized animal is fixed at the site of injection where the contact of antigen and antibody causes an acute inflammatory reaction (Arthus phenomenon). In view of this work on immunized animals and of the results obtained with trypan blue and iron, experiments were undertaken to determine whether a readily identified foreign protein such as horse serum injected into an area of inflammation caused by either bacteria or a sterile irritant would also be retained *in situ* by the inflammatory reaction.

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Penetration of Horse Serum into the Blood Stream after Its Injection into the Inflamed Peritoneal Cavity

Horse serum varying in quantity from 2 to 8 cc. was injected into the peritoneal cavity of normal rabbits and of rabbits that had previously been given an intraperitoneal injection of an inflammatory irritant. Sterile inflammation was induced by a mixture of 5 per cent of aleuronat and 3 per cent of starch in 0.5 per cent saline solution. This mixture was injected into the peritoneal cavity 24 to 48 hours prior to the injection of horse serum.

Samples of blood were removed from the heart at varying intervals after the intraperitoneal injection of horse serum. The blood was centrifugalized and the serum tested for horse serum by anti-horse-serum obtained from rabbits repeatedly injected with this foreign protein. The blood serum was diluted by the addition of 2 cc. of saline to 1 cc. of serum. Varying dilutions of blood serum in multiples of 3 were then made from this initial dilution. The final tests were performed by the addition to 0.7 cc. of saline of 0.1 cc. of the diluted blood serum and 0.2 cc. of anti-horse-serum.

Tables I, II, and III summarize all the experiments by giving the results of tests for presence of horse serum in the blood at varying intervals after intraperitoneal injection of the foreign protein. These tests serve as a rough measure of the concentration of the antigen in the blood serum. A few illustrative experiments follow.

Experiment 10.—Rabbit 17 was injected with 4 cc. of horse serum when an inflammatory reaction had been in progress for about 48 hours. Rabbit 8 served as control receiving 4 cc. of horse serum intraperitoneally. The results of tests for presence of horse serum in the blood 2 hours after its injection appear in Table I. The results of tests 6 hours and 24 hours later are shown in Tables II and III respectively. Following the introduction of horse serum into the inflamed peritoneal cavity its concentration in the blood is conspicuously less after 2 and 6 hours than that found after injection into the normal peritoneal cavity; but after 24 hours the horse serum in the blood of both animals has the same concentration.

In the following experiment samples of blood were removed from the heart about 6 hours after injection of horse serum into the normal and inflamed peritoneal cavity of rabbits.

Experiment 13.—4 cc. of horse serum were injected into the peritoneal cavity of Rabbit 20. Rabbit 23, in which peritonitis had been induced 24 hours previously, received the same amount of horse serum. 5½ hours later samples of blood were removed from the heart and tested for the presence of horse serum, as already described. The results appear in Table II.

TABLE I

Presence of Horse Serum in the Blood Stream About 2 Hours after Intraperitoneal Injection

Experiment No.	Duration of inflammation (hours)	Animals with inflamed peritoneal cavity								Animals with normal peritoneal cavity							
		Dilutions								Dilutions							
		1:3	1:9	1:27	1:81	1:243	1:729	1:2187	1:6561	1:3	1:9	1:27	1:81	1:243	1:729	1:2187	1:6561
1	24	Faint trace	Faint trace?	0	0	0	0	0	0	++	+	+	Trace	Faint trace	0	0	0
2	26	+++	++	+	+	Trace	Faint trace	0	0	+++	+++	++	+	Trace	Trace	+	+
3	26	++	++	+	0	0	0	0	0	+++	++	++	Trace	Faint trace	Faint trace	+	0
4	26	+	Trace	0	0	0	0	0	0	++	+	+	Trace	0	0	0	0
5	26	+	0	0	0	0	0	0	0	++	+	+	0	0	0	0	0
6	49	0	0	0	0	0	0	0	0	++	+	+	+	+	0	0	0
7	50	0	0	0	0	0	0	0	0	+++	++	+	Trace	0	0	0	0
10	49	+	Trace	0	0	0	0	0	0	++	+	+	Trace	0	0	0	0
11	50	++	+	Trace	0	0	0	0	0	++	+	+	Trace	0	0	0	0

TABLE II

Presence of Horse Serum in the Blood Stream 5 to 7 Hours after Intraperitoneal Injection

Experiment No.	Duration of inflammation (hours)	Animals with inflamed peritoneal cavity							Animals with normal peritoneal cavity						
		Dilutions							Dilutions						
		1:3	1:9	1:27	1:81	1:243	1:729	1:2187	1:3	1:9	1:27	1:81	1:243	1:729	1:2187
13	30	++	+	Trace	0	0	0	0	+++	++	+	+	Trace	0	0
5	31	++	+	Trace	0	0	0	0	+++	++	+	Trace	0	0	0
12	33	Trace	0	0	0	0	0	0	++	+	Trace	0	0	0	0
15	52	+	+	Trace	0	0	0	0	++	++	+	Trace	0	0	0
11	54	++	+	Trace	0	0	0	0	++	+	Trace	Faint trace?	0	0	0
10	55	+++	+	Trace	0	0	0	0	+++	++	+	Trace	0	0	0

TABLE III

Presence of Horse Serum in the Blood Stream 24 to 30 Hours after Intraperitoneal Injection

Experiment No.	Duration of inflammation (hours)	Animals with inflamed peritoneal cavity							Animals with normal peritoneal cavity						
		Dilutions							Dilutions						
		1:3	1:9	1:27	1:81	1:243	1:729	1:2187	1:3	1:9	1:27	1:81	1:243	1:729	1:2187
4	49	+++	++	+	+	Trace	0	0	+++	++	+	+	Trace	0	0
5	50	++	+	+	Trace	0	0	0	+++	++	+	0	0	0	0
8	50	++	++	+	Trace	Trace	0	0	++	++	++	+	Trace	0	0
9	50	++	++	+	Trace	0	0	0	+++	+++	+	+	Trace	0	0
10	70	+++	++	+	Trace	0	0	0	+++	++	+	Trace	0	0	0
11	71	++	+	Trace	0	0	0	0	++	+	0	0	0	0	0
6	78	0	0	0	0	0	0	0	+++	+++	++	Trace	0	0	0

From this experiment it is evident that 5 hours and 30 minutes after injection of horse serum into a peritoneal cavity that has been inflamed for 24 hours the penetration of the foreign protein into the blood stream is delayed.

A similar experiment in which the inflammatory process had been going on for 48 hours before the injection of foreign protein follows.

Experiment 15.—Rabbit 21 received 4 cc. of horse serum intraperitoneally; 5 hours later 3 cc. of blood were removed from the heart. Rabbit 24 was injected with the same amount of horse serum 47 hours after receiving 10 cc. of the aleuronat suspension intraperitoneally. 5 hours after the injection of horse serum 3 cc. of blood were withdrawn from the heart. Tests for the presence of horse serum in the blood of both of these rabbits yielded the results shown in Table II.

It is clear that there has been a definite retardation in the passage of the protein after its injection into an inflamed peritoneal cavity.

Samples of blood withdrawn from the heart 24 to 30 hours after injection of horse serum into both normal and inflamed peritoneal cavities showed in most instances no appreciable differences (Table III).

Experiment 4.—4 cc. of horse serum were injected into the peritoneal cavity of Rabbit 13, 24 hours after aleuronat had been injected into the cavity. 2 hours later this animal was bled from the heart. Rabbit 4 which served as control received intraperitoneally 4 cc. of horse serum and was bled 2 hours later. The results of this experiment appear in Table I. It is seen that after injection of protein into the inflamed peritoneal cavity its concentration in the circulating blood is distinctly lower than after injection into the normal cavity. A second sample of blood was removed 25 hours after the injection of horse serum.

The presence of horse serum in the blood stream 1 day after intraperitoneal injection of the protein is recorded in Table III. Although there was delay in the penetration of horse serum into the blood stream 2 and 6 hours after injection into an inflamed peritoneal cavity, the concentrations of horse serum in the blood of experimental and control animals are now equal.

The Retention of Horse Serum at the Site of Inflammation

In the above experiments it was shown that when horse serum is injected into an inflamed peritoneal cavity its passage into the blood

stream is delayed during several hours. To determine whether this delay is due to retention of the foreign protein in the inflamed peritoneal cavity a series of experiments was undertaken to measure directly changes in the quantity of horse serum at the site of inflammation.

10 cc. of 5 per cent aleuronat and 3 per cent starch in 0.5 per cent saline were injected into the peritoneal cavity of rabbits. 24 to 48 hours later 2 to 4 cc. of horse serum were injected into the inflamed peritoneal cavity. Each experiment was controlled by the injection of the same amount of horse serum into the peritoneal cavity of a normal rabbit. 2 to 5 hours later 10 cc. of saline were injected into both normal and inflamed peritoneal cavities. This was done in order to be certain of obtaining a sufficiently large sample of fluid for the tests. The rabbits were then immediately bled from the heart and the abdomen was opened under anesthesia with ether. The peritoneal fluid was tested for the presence of horse serum. A typical experiment follows.

Experiment 11.—Rabbit 9 received an injection of 2 cc. horse serum intraperitoneally. 2½ hours later 10 cc. of saline were injected into the peritoneal cavity. Immediately afterwards 4 cc. of blood were withdrawn from the heart. About 3 cc. of serous peritoneal fluid were obtained. The same technique was followed in Rabbit 18, which had, however, received an intraperitoneal injection of 10 cc. of aleuronat suspension 47 hours before. Tests for the presence of horse serum in the blood are given in Table I. Tests for the presence of horse serum at the site of inflammation are shown in Table IV.

Essentially the same results were obtained in the following experiment when the horse serum was injected 5 hours previous to the removal of the samples of blood and peritoneal fluid.

Experiment 15.—4 cc. of horse serum were injected into the normal peritoneal cavity of Rabbit 21; 5 hours later 10 cc. of saline were injected into the cavity and immediately afterwards 3 cc. of blood were withdrawn from the heart. About 1 cc. of serous fluid was then obtained from the peritoneal cavity. Rabbit 24 was given an injection of 10 cc. aleuronat suspension into the peritoneal cavity and 47 hours later 4 cc. of horse serum. 5 hours later samples of peritoneal fluid and blood were obtained as described for Rabbit 21. The tests for presence of horse serum at the site of inflammation are shown in Table IV, and in the blood stream in Table II.

It is evident from these experiments that there is a greater retention of horse serum *in situ* when the foreign protein is injected into an inflamed peritoneal cavity than when injected into a normal one. This observation is correlated with the delay in the penetration of the protein into the blood stream.

It has been seen (1) that when the inflammatory reaction was in-

TABLE IV

Retention of Horse Serum at Site of Inflammation

Experiment No.	Interval between injection of horse serum and tests for its presence (hours: minutes)	Total duration of inflammation (hours)	Animals with inflamed site previous to injection of horse serum							Animals with normal site previous to injection of horse serum						
			Dilutions							Dilutions						
			1:3	1:9	1:27	1:81	1:243	1:729	1:2187	1:3	1:9	1:27	1:81	1:243	1:729	1:2187
14	2:30	26	+	++	+	+	Trace	0	0	+	Trace	0	0	0	0	0
11	2:30	50	+++	++	+	Trace	Trace	0	0	++	+	+	0	0	0	0
15	5:00	52	+++	++	+	+	+	Trace	0	++	+	+	Trace	0	0	0
16*	24:00	42	+	++	+	Trace	Trace	0	0	+	Trace	0	0	0	0	0
17*	24:00	48	++	+	Faint trace	0	0	0	0	+	0	0	0	0	0	0

* Site of injection of horse serum and inflammation was located in skin.

duced in the peritoneal cavity of rabbits the fixation of trypan blue was less effective than when the subcutaneous tissue was the site of the experimental inflammation. For this reason horse serum was injected into an inflamed area on the skin of the abdomen.

About 0.2 cc. of a saline suspension of *Staphylococcus aureus* was injected intracutaneously. 24 hours later 0.15 cc. of horse serum was injected directly into the inflamed area, which was removed 24 hours later, weighed and cut into small pieces. These were thoroughly ground in a mortar with a volume of saline equal to 10 times the weight of the dermal area. The extract was centrifugalized at rapid speed for about 45 minutes. In the control experiment 0.15 cc. of horse serum was injected into the normal skin of a rabbit and the tissues were extracted in saline 24 hours later. The results of two such experiments are shown in Table IV.

Retention of the foreign protein is evident 24 hours after its injection into an area of cutaneous inflammation.

These experiments show that like trypan blue and iron, complex foreign proteins such as are found in horse serum when injected into an inflamed area are retained *in situ* by the inflammatory reaction and penetrate less rapidly into the circulating blood stream than when injected into normal tissue. Opie (4, 5), as has been pointed out, showed that when foreign protein is first injected into the skin of a normal animal it rapidly leaves the site of injection and enters the circulating blood. In an animal that has been immunized reinjection with the foreign protein produces an intense inflammatory reaction with necrosis at the point of entry. The foreign protein is fixed at the site of injection, and fails to enter the circulating blood. The results that the writer has obtained are in agreement with these observations and show that an inflammatory reaction (caused in Opie's experiments by the contact of antigen and antibody and in these experiments by a sterile irritant) arrests the protein at the point of entry.

The Accumulation of Horse Serum in Inflamed Cutaneous Areas

Having demonstrated that the penetration of horse serum into the blood stream is delayed at the site of inflammation the attempt was made to determine whether horse serum injected into the circulating blood stream would accumulate in inflamed areas to a greater extent

TABLE V

The Accumulation of Horse Serum in Inflamed Cutaneous Areas

Experiment No.	Inflamed skin							Normal skin						
	Dilutions							Dilutions						
	1:3	1:9	1:27	1:81	1:243	1:729	1:2187	1:3	1:9	1:27	1:81	1:243	1:729	1:2187
21	+	+	Trace	Faint trace	0	0	0	Faint trace	0	0	0	0	0	0
22	++	+	Trace	0	0	0	0	Trace	0	0	0	0	0	0
23	+	Trace	0	0	0	0	0	Trace	0	0	0	0	0	0
24	+++	++	+	Trace	0	0	0	+	Trace	Faint trace	0	0	0	0
25	++	+	+	Trace	0	0	0	+	+	Trace	0	0	0	0
26	++	++	+	Trace	0	0	0	++	+	Trace	0	0	0	0
27	++	++	+	Trace	0	0	0	+	+	Trace	0	0	0	0
28	+++	++	+	Trace	0	0	0	++	+	0	0	0	0	0
29	+++	++	+	0	0	0	0	++	+	+	0	0	0	0
30	++	+	+	0	0	0	0	+	Trace	0	0	0	0	0

than in normal tissue, as had been observed with trypan blue and ferric chloride.

Areas of cutaneous inflammation were induced by the injection of about 0.2 cc. of a saline suspension of *Staphylococcus aureus* into the skin of the abdomen of rabbits. About 3 hours later 10 cc. of horse serum were injected intravenously. The animal was killed when the inflammation was of 5 to 6 hours duration. The inflamed areas were removed and weighed. They were then cut into thin strips and ground in a mortar with a volume of saline equal to twice the weight of the tissue. In earlier experiments the extracts were obtained by grinding with saline alone, but in subsequent experiments by means of sand and saline. As control areas, strips of normal skin from the abdomen of the same rabbit were removed and the foreign protein extracted by the same technique as with the inflamed areas. The extracts were centrifugalized at rapid speed for 45 minutes. After the top layer of fat was carefully removed, 1 cc. of the extract was drawn off with a pipette into a test tube containing 2 cc. of saline. Dilutions of the extract in multiples of 3 were then prepared from this first tube. In the final test 0.1 cc. of diluted extract was added to 0.2 cc. of anti-horse-serum and the mixture made up to 1 cc. with saline. The final dilutions were kept 1 hour in a water bath at 38°C. and then placed overnight on ice. Readings were made on the following morning. The results of all the experiments are shown in Table V.

In all the experiments there is consistently a greater concentration of the foreign protein in inflamed than in normal tissue. These results are therefore similar to those obtained with trypan blue and with ferric chloride. The accumulation of foreign protein in inflamed tissue is doubtless in part the result of the increased passage of fluid from the circulating blood stream, but observations recorded above indicate that its escape from the site of inflammation is retarded.

Some years ago Auer (6) showed that if the ear of a rabbit immunized with horse serum is painted with xylol, reinjection of the antigen causes in the affected ear an intense inflammatory reaction followed by necrosis. No such severe reaction was seen when xylol had been applied to the ear of a normal rabbit after a single injection of horse serum. In view of the observations on the accumulation of foreign protein in inflamed areas it is highly probable that the reaction in Auer's experiment is simply the result of an accumulation from the circulating blood stream of antigen and antibody in the inflamed ear of sensitized animals; the contact of antigen and antibody in the tissues

causing an acute inflammatory reaction and intensifying thus the mild reaction caused by xylol alone.

A few years ago Opie (personal communication) repeated the experiments of Auer with crystalline egg albumin and instead of applying xylol as an inflammatory irritant, he dipped the ear of the experimental animal into water at 50°C. for 5 minutes. On the following day crystalline egg albumin was injected into the peritoneal cavity of the animal. Several days later necrosis of the inflamed ear was observed in many instances.

Experiment 31.—Rabbit 30 received 5 injections of 1 cc. horse serum administered subcutaneously at 5 to 6 day intervals. 19 days after the last injection 10 cc. of horse serum were injected into the peritoneal cavity. 1 hour later an ear of this animal was rubbed lightly for a minute with a pledget of cotton soaked in xylol. 2 days later the ear showed only a very mild reaction. It was then placed in water at 54°C. for 5 minutes. It immediately flushed. 10 cc. of horse serum were then reinjected into the peritoneal cavity. 24 hours later the ear was very much congested and edematous, revealing a pronounced inflammatory reaction.

The animal was etherized and bled to death. The inflamed and the normal ear were removed. The weight of the inflamed ear was 13.5 gm. and of the normal ear 5 gm. Each ear was then cut into thin strips and ground separately in a mortar with a volume of saline equal to its own weight. The extracts were then centrifugalized at rapid speed for 40 minutes and the supernatant fluid tested for the presence of horse serum. As control to this experiment, Rabbit 31, which had not been sensitized to the foreign protein, was treated in the same manner as Rabbit 30. The results of this experiment are shown in the following table:

Dilutions of skin extract	Immunized Rabbit 30		Normal Rabbit 31	
	Normal ear	Inflamed ear	Normal ear	Inflamed ear
1:3	++	+++	Trace	++
1:9	Trace	++	Faint trace	+
1:27	0	+	0	Faint trace
1:81	0	+	0	0
1:243	0	0	0	0
1:729	0	0	0	0
1:2187	0	0	0	0

When xylol alone was used as the inflammatory irritant, essentially the same results were obtained.

Experiment 32.—Rabbit 32 received 5 injections of 1 cc. horse serum administered subcutaneously at 3 to 5 day intervals. 5 days after the last injection 10 cc.

of horse serum were injected into the peritoneal cavity. 1½ hours later the right ear was rubbed gently with 2 cc. of xylol for about 2 minutes. The application of xylol was repeated twice in the next 2 hours at 1-hour intervals. 24 hours later the ear was mildly edematous and congested, with a few blisters.

The animal was killed under ether and both ears were removed. The weight of the inflamed ear was 3.7 gm. and of the normal ear 2.2 gm. Each ear was cut into thin strips and ground separately in a mortar with a volume of saline equal to twice its own weight. The extracts were then centrifugalized at rapid speed for 1½ hours. After being filtered, the supernatant fluid was tested for the presence of horse serum. The samples containing the extract in a dilution of 1 to 3 were not read owing to slight cloudiness in the control tubes in which anti-horse-serum was absent. As control to this experiment, Rabbit 33, which had not been sensitized to the foreign protein, was treated in the same manner as Rabbit 32. The results of this experiment are shown in the following table:

Dilutions of skin extract	Immunized Rabbit 32		Normal Rabbit 33	
	Normal ear	Inflamed ear	Normal ear	Inflamed ear
1:3			+	++
1:9	+	++	+	++
1:27	Faint trace	+	0	+
1:81	0	Trace	0	Faint trace
1:243	0	0	0	0
1:729	0	0	0	0
1:2187	0	0	0	0

These experiments show that horse serum accumulates from the circulating blood in the inflamed ear in greater concentration than in the normal ear. The severe inflammatory reaction in the ears of the sensitized animals in the experiments of Auer, when the same antigen was reinjected, was doubtless due to the accumulation and contact in the inflamed tissues of antigen and antibody from the circulating blood.

It is possible that the phenomenon of focal reaction in tuberculosis can be explained by the observations reported in this paper. When tuberculin is introduced into the blood stream of an animal with a tuberculous lesion, an intense inflammatory reaction may develop in the lesion. The mechanism of this focal reaction in tuberculosis is not understood. It is conceivable in view of the above experiments with horse serum that in a like manner tuberculo-protein may accumulate

from the blood stream in the inflamed tuberculous lesion and by its presence there induce a local inflammatory reaction.

CONCLUSIONS

Foreign protein such as horse serum injected into an inflamed peritoneal cavity penetrates into the blood stream less rapidly than when introduced into the normal cavity.

Foreign protein injected into a cutaneous inflammatory area is held *in situ* for a longer period than when injected into an inflamed peritoneal cavity.

Foreign protein introduced into the circulating blood stream accumulates in an inflamed area, where it is found in greater concentration than in normal tissue.

Accumulation of foreign protein at the site of inflammation explains the phenomenon of local anaphylaxis described by Auer in rabbits sensitized to horse serum. The antigen accumulating in the inflamed ear reacts with antibody, intensifies a pre-existing inflammatory reaction and produces necrosis of the ear.

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SUSCEPTIBILITY OF RABBITS TO INFECTION BY THE INHALATION OF TYPE II PNEUMOCOCCI

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(Received for publication, April 21, 1930)

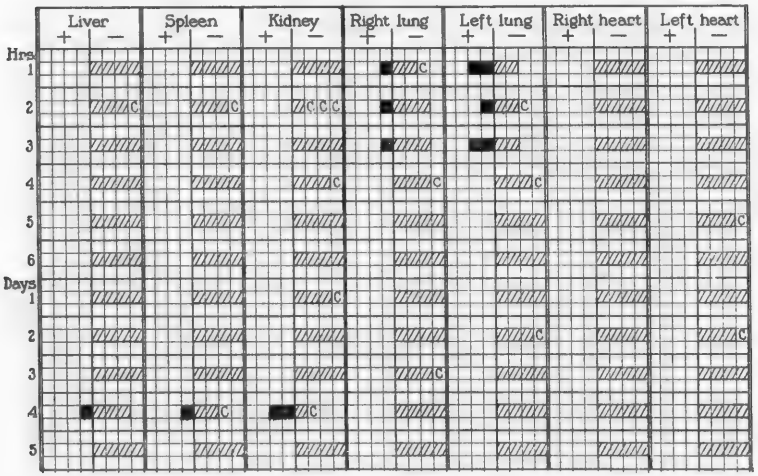
In preceding papers (1) it has been shown that following inhalation, virulent Type I pneumococci generally disappear from the lungs of rabbits within a few hours, only to reappear later in certain instances in which a general septicemia occurs. It has further been shown that some animals may recover from a Type I pneumococcus septicemia. Rabbits are, nevertheless, very susceptible to infection by Type I pneumococci. In order to determine how they would react to less virulent strains of pneumococci, Type II pneumococcus was selected. Three different strains of Type II were used. One strain was converted into the "R" form, by repeated transfers in Type II anti-pneumococcus serum. The changes from the "S" to the "R" form are accompanied by loss of capsule, type specificity, and virulence for mice. This strain is designated Pneumococcus IIR and is avirulent for rabbits. The original Pneumococcus Type II strain is designated Pneumococcus Type II (SAv). An intraperitoneal injection of 0.1 cc. of a broth culture of this stock strain is necessary to kill rabbits. It was rendered rabbit virulent by rapid passage. This virulent strain, called Pneumococcus II (Sv), regularly kills rabbits following intraperitoneal injections of 0.000,01 cc.

In the present paper are reported (1) the distribution of pneumococci in the organs of rabbits following exposures to repeated inhalations of the above mentioned strains, (2) the occurrence of positive blood cultures, and (3) the mortality of the rabbits exposed to each of the above mentioned strains.

Method

The rabbits were placed in a large spray chamber similar to that already described (2) and exposed to a spray of pneumococci. 150 cc. of an 18 hour broth

culture was used for each spraying. The animals were exposed at 10 day intervals. Before each spraying, a sample of blood was obtained by bleeding the animal from the ear vein. At daily intervals after the first spraying, bood cultures were taken from a few of the rabbits. In the case of the animals which were killed and autopsied, broth cultures were made from both sides of the heart, and small pieces of the periphery of both lungs, kidney, liver, and spleen were placed directly in tubes of broth. Heart's blood cultures alone were made from the rabbits which died. All cultures were plated on blood agar for further identification. The animals that were killed were first chloroformed, immersed in a solution of lysol, and autopsied with sterile instruments.



TEXT-FIG. 1. Distribution of IIR pneumococci in organs following spraying. The black squares indicate organs in which cultures showed pneumococci to be present, the cross-hatched squares represent organs in which cultures remained sterile. "c" = contamination.

Distribution of Pneumococci IIR in the Organs

In Text-fig. 1 is shown the distribution of IIR pneumococci in the organs of 44 sprayed rabbits which were killed at intervals after spraying. One to 6 hours after spraying pneumococci were only recovered from the periphery of the lungs in less than half of the animals sacrificed within the first 3 hours. Pneumococci were never recovered in this group from the liver, spleen or kidney. The heart's

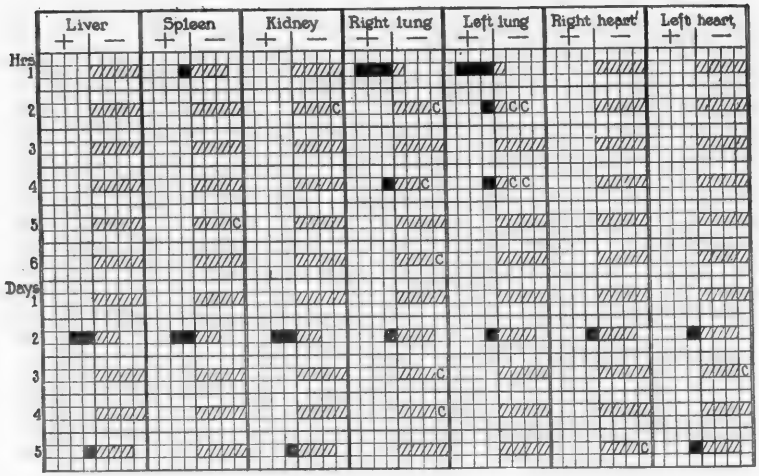
blood cultures from these animals remained sterile. Of the remaining 20 rabbits killed from 1 to 5 days following exposure, pneumococci were only recovered from 2 rabbits killed on the 4th day. In one of these rabbits pneumococci were recovered from the liver, kidney and spleen and in another only from the kidney. This experiment shows how readily rabbits destroy the non-virulent R-forms of pneumococcus. These organisms were never recovered from the blood, rapidly disappeared from the lungs, and were rarely recovered from other viscera.

Daily blood cultures were taken from 7 rabbits following their first exposure to a spray of Pneumococcus IIR. During the 10 days period of observation in no instance were pneumococci recovered from the blood.

In all, 17 rabbits were exposed to from 3 to 10 inhalations of avirulent Type IIR pneumococci. Eight rabbits completed the course of 10 exposures. No rabbit became infected.

Distribution of Pneumococci Type II (SAv) in the Organs

In Text-fig. 2 is shown the distribution of the slightly virulent Pneumococci Type II (SAv) in the organs of 44 sprayed rabbits which were killed at intervals after spraying. It will be seen that pneumococci were recovered from the lungs of 3 of the 4 rabbits killed in 1 hour and that although the heart's blood cultures remained sterile, in one instance pneumococci were demonstrated in the culture from the spleen. After the 1st hour, however, the pneumococci were only recovered from the lungs in 3 instances. Of the remaining 20 rabbits killed from 1 to 5 days following exposure, 1, or 5 per cent, showed a general pneumococcus septicemia on the 2nd day. Although in the other rabbits pneumococci were not recovered from the lungs, in 2 instances the liver and kidney contained organisms and the spleen was positive in 1 rabbit. From the left heart's blood of one of the rabbits killed on the 5th day a positive culture was obtained but the culture of the right heart and spleen of this animal remained sterile. This experiment shows that a few rabbits may develop a generalised septicemia following inhalation of the slightly virulent Pneumococcus Type II (SAv).



TEXT-FIG. 2. Distribution of Type II (SAv) pneumococci in organs following spraying. The black squares indicate organs in which cultures showed pneumococci to be present; the cross-hatched squares represent organs in which cultures remained sterile. "c" = contamination.

TABLE I

Days on Which Positive Blood Cultures Were Obtained Following a Single Exposure to Inhalation of Pneumococci Type II (SAv)

Rabbit No.	Days										Course
	1	2	3	4	5	6	7	8	9	10	
1	−	−	−	−	c	−	−	−	−	−	Survived
2	c	−	−	−	c	c	−	−	+	+	"
3	−	−	−	−	−	−	−	−	+	−	"
4	+	+	−	−	−						Died 5th day. Blood sterile
5	−	+	−	−	−	−	−	−	−	−	Survived
6	−	−	−	−	−	−	−	−	−	−	"
7	−	−	−	−	−	−	−	−	−	−	"

+ indicates a positive culture of pneumococcus; − = a sterile culture; and "c" = a contaminated culture.

Blood Cultures

Daily blood cultures were taken from 7 rabbits following their first exposure to a spray of Pneumococcus II. From Table I it is seen that positive blood cultures were obtained from 4 of these rabbits. In 2 instances the blood was positive on but one occasion. In the other 2 rabbits the blood cultures were positive on 2 successive days. One of these rabbits survived, the other rabbit died. As the latter rabbit's blood had been sterile for 3 days and as the postmortem blood culture remained sterile, it is evident that death was not due to pneumococcus septicemia.

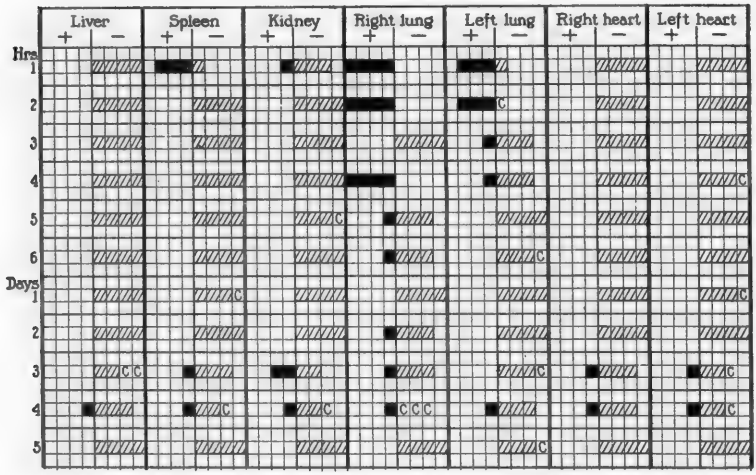
In all, 11 rabbits were exposed to from 1 to 10 inhalations of slightly virulent Type II pneumococci (SAv). Eight rabbits completed the course of 10 exposures. All these rabbits showed no evidence of infection.

Distribution of Rabbit Virulent Strain of Type II Pneumococcus (Sv)

In Text-fig. 3 is shown the distribution of rabbit virulent Type II (Sv) pneumococci in the organs of 44 sprayed rabbits which were killed at intervals after spraying. From the 24 rabbits killed in from 1 to 6 hours after their initial exposure, pneumococci were generally recovered from the periphery of the lungs for the first 2 hours but later they rapidly disappeared. During this same period pneumococci were only recovered in 3 rabbits from the spleen and in 1 rabbit from the kidney. Of the remaining 20 rabbits killed from 1 to 5 days following exposure, 2 or 10 per cent showed a general pneumococcus septicemia. In 2 other rabbits pneumococci were recovered from the lungs, and in 1 animal from the kidney. The results of this experiment do not markedly differ from those obtained in the case of the rabbits exposed to the slightly virulent strain of Pneumococcus Type II (SAv). A few rabbits may become infected following inhalations of rabbit virulent strain of Type II pneumococci (Sv). It further indicates that these virulent pneumococci do not disappear from the lung or organs as rapidly as do the avirulent organisms. If they persist, however, a generalised septicemia is more apt to be present.

Blood Cultures

Blood cultures were taken from 11 rabbits following their first exposure to a spray of rabbit virulent Pneumococcus Type II (Sv).



TEXT-FIG. 3. Distribution of Type II (Sv) pneumococci in organs following spraying. The black squares indicate organs in which cultures showed pneumococci to be present, the cross-hatched squares represent organs in which cultures remained sterile. "c" = contamination.

TABLE II

Days on Which Positive Blood Cultures Were Obtained Following a Single Exposure to a Pneumococcus II (Sv)

Rabbit No.	Days										Course
	1	2	3	4	5	6	7	8	9	10	
1	−	−	−	−	c	+	+				Died 8th day
2	−	c	−	−	−	−	−	−	−	−	Survived
3	c	−	−	−	−	−	−	−	−	−	"
4	−	−	−	−	−	−	−	−	+	−	"
5	−	−	−	−	−	−	−	c	−	−	"
6	+	+	+								Died 4th day
7	−	−	−	−	−	−	−	−	−	−	Survived
8	−	c	+	c	−	−	−	−	−	−	"
9	−	−	−	−	−	−	−	−	−	−	"
10	+	c	−	−	−	−	−	−	−	−	"
11	−	+	+	+	−	−	−	−	−	−	"

+ indicates a positive culture of pneumococcus; − = a sterile culture; and "c" = a contaminated culture.

As is seen from Table II, of the 6 rabbits which developed septicemia 2 or 18 per cent continued to have a positive blood culture until death. The other 4 rabbits had merely a transient septicemia and survived. In some instances, the septicemia could be detected during the first 24 hours, but in others the organisms were first recovered as late as the 6th or 9th day.

TABLE III

Fate of Rabbits after One to Ten Exposures to Inhalation of Rabbit Virulent Type II Pneumococcus*

No. of times exposed to virulent pneumococci	No. of rabbits exposed	No. dying	Pneumococcus recovered from heart's blood	Pneumococcus not recovered from heart's blood
1	50	14	12	2
2	36	1	—	1
3	34	2	1	1
4	30	1	—	1
5	26	2	1	1
6	24	2	1	1
7	21	—	—	—
8	17	1	—	1
9	17	—	—	—
10	16	1	1	—

* The discrepancy between the total numbers and the sum of the numbers in the individual groups is due to the fact that certain of the animals that survived were used for intraperitoneal tests or for other experiments.

Mortality of Rabbits Following Exposure to Inhalation of Pneumococcus Type II (Sv)

Table III shows the number of normal rabbits which succumbed during the course of spraying with rabbit virulent Pneumococcus II (Sv). From this table it is seen that out of 50 rabbits 15 or 28 per cent died following the first exposure. Pneumococci were recovered from the heart's blood in 12 instances. Out of the total of 24 rabbits which died, in 16 instances the organisms were recovered from the heart's blood, and the cultures remained sterile in the other 8. Although care was exercised to open the spray boxes promptly 1 hour after spraying, these animals probably died of suffocation. This table further shows that the greatest number of deaths occur after the first exposure. It is

difficult to explain, however, how some rabbits which have successfully survived 5, or even 9, exposures finally become infected.

The interval elapsing between the time of spraying and death from pneumococcus septicemia is shown in Table IV. From this it is seen that there is great irregularity in the time of death following the initial exposure. In fact 4 rabbits only succumbed on the 10th day. It is interesting to observe that in rabbits which had survived the first exposure, infection was delayed and death occurred from the 7th to 10th day after spraying.

In no instance was there any gross evidence of pneumonia, although serous and serofibrinous pleurisy and serofibrinous pericarditis were common.

TABLE IV

Number of Days Elapsing between Spraying and Death of Rabbits from Pneumococcus Type II (Sv) Septicemia

	Days									
	1	2	3	4	5	6	7	8	9	10
Spray I	—	1	2	2	—	1	1	1	—	4
" III	—	—	—	—	—	—	—	—	1	—
" V	—	—	—	—	—	—	1	—	—	—
" VI	—	—	—	—	—	—	1	—	—	—
" X	—	—	—	—	—	—	—	—	1	—

DISCUSSION

From the foregoing experiments it appears that the susceptibility of rabbits to infection following inhalation of the same type of pneumococcus is in direct proportion to the virulence of the strain used.

The non-virulent IIR pneumococci may be recovered from the periphery of the lungs for a period of 3 hours after spraying. After this time they are only occasionally recovered from any part of the body. The slightly virulent Type II (SAv) and virulent Type II (Sv) may likewise be recovered from the lungs for a few hours following exposure. But in the case of the more virulent strains, there appears to be an almost immediate general dissemination of the organisms throughout the body, as is evidenced by the recovery of pneumococci from the

spleen. The organisms then disappear from the blood only to reappear after 48 hours. This reappearance is often in the form of a septicemia. The same phenomenon was noticed in the case of rabbits sprayed with virulent Type I pneumococci. It is unfortunate that the subsequent penetration of the inhaled pneumococci into the pulmonary tissues cannot be followed. But the great number of other organisms which normally inhabit the bronchi of rabbits, as Jones (3) has shown, renders cultural studies of the lungs difficult. Histological detection of pneumococci in the tissues is also unreliable when the organisms are present only in small numbers.

In all probability the majority of the organisms are destroyed either where they are first implanted or deeper in the lung tissue by the endothelial leucocytes or by poly-morphonuclear leucocytes. In any case, a few organisms occasionally filter through into the blood stream. Gaskell (4) believes that invasion of the blood stream by pneumococci probably always takes place in the early stages of an air-borne infection of the lung. The actual invasion of the blood stream must be much more common than is generally supposed. Not only are there probably few organisms free at any one time in the circulating blood but these few bacteria may even be within leucocytes and not in reality multiplying in the blood.

The occasional organisms which reach the blood are probably rapidly filtered out of the blood stream by the organs and locally destroyed. It is evident, however, that following the apparent disappearance of the pneumococci from the periphery of the lungs, they again appear, this time in the blood, and as a result may be recovered from all the organs. The point of this reinvasion is as yet uncertain.

The results of the blood cultures taken following the initial spraying parallel the distribution of pneumococci in the rabbits which were sacrificed. The cultures from the rabbits sprayed with avirulent degenerated non-virulent "R" forms pneumococci remained sterile; an occasional positive culture was obtained from the animals exposed to the slightly virulent Type II (SAv) organisms but no animals succumbed. Only in the case of the rabbits exposed to inhalations of rabbit virulent Type II (Sv) pneumococci did the more susceptible rabbits die of an overwhelming septicemia. But some rabbits were even able to overcome the septicemia following exposure to this rabbit virulent strain.

Following the initial spraying with a highly rabbit virulent Type II (Sv) pneumococcus the majority of susceptible rabbits died of an overwhelming septicemia without any attempt on the part of the body to localize the infection.

SUMMARY

1. The susceptibility of rabbits to inhalations of pneumococci varies in direct proportion to the virulence of the organism for rabbits.

2. When rabbits are exposed to a pneumococcus spray, irrespective of the virulence of the organism, the bacteria readily penetrate into the lower respiratory tract.

3. When rabbits are exposed to a spray of avirulent degenerated "R" form of pneumococcus, septicemia does not occur and pneumococci are seldom recovered from the liver, kidney or spleen.

4. When rabbits are exposed to a spray of slightly virulent Pneumococcus Type II (SAv) a non-fatal septicemia may occur and pneumococci may be recovered from the liver, kidney and spleen.

5. When rabbits are exposed to a spray of virulent Type II (Sv) pneumococci, septicemia may occur which in certain instances terminates fatally. Pneumococci may also be recovered from the liver, kidney and spleen.

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DEVELOPMENT OF AGGLUTININS AND PROTECTIVE ANTIBODIES IN RABBITS, AFTER INHALATION OF TYPE II PNEUMOCOCCI

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(Received for publication, May 9, 1930)

In preceding papers (1) it has been shown that mice and rabbits may acquire a high degree of immunity, following repeated inhalations of living pneumococci. In this work it was shown that the strain of Type I pneumococcus used produced a high mortality among the exposed rabbits and that many of the survivors have agglutinins and protective antibodies in their serum. In the present study it seemed interesting to determine the immunity response to less virulent strains. For this purpose a Type II pneumococcus was used. Pneumococcus Type II, however, is relatively avirulent for rabbits. One strain was transformed into the "R" form by repeated transfers in antipneumococcus Type II serum. The changes to the "R" form are accompanied by loss of capsule, type specificity, and virulence. This strain is referred to as Pneumococcus IIR. The stock culture of Pneumococcus Type II would cause a fatal septicemia in rabbits when injected intraperitoneally in doses of at least 0.1 cc. This strain is called Pneumococcus Type II (SAv). By repeated animal passages the virulence of this strain was raised so that an intraperitoneal injection of 0.000,01 cc. of broth culture would regularly kill rabbits. This virulent culture is designated as Pneumococcus Type II (Sv).

It has already been shown that rabbits are only susceptible to fatal infection by inhalations of virulent Type II pneumococci (Sv) (2). Although pneumococcus septicemia may be caused in an occasional rabbit following inhalation of the slightly virulent Pneumococcus Type II, death does not ensue. In the present paper is reported the development of (1) agglutinins and (2) protective antibodies in the blood serum of rabbits following repeated inhalations of aviru-

lent IIR, slightly virulent Type II (SAv), and virulent Type II (Sv) pneumococci.

Method

Rabbits were placed in a large spray chamber similar to that already described (3) and exposed to a spray of pneumococci. 50 cc. of an 8 hour broth culture were used for each spraying. The animals were exposed at 10 day intervals. Before each spraying, a sample of blood was obtained from the ear vein of each animal. None of the normal rabbit sera contained agglutinins nor did the normal sera protect mice against intraperitoneal injections of 0.000,001 cc. of Type II pneumococcus.

10 days after each exposure the presence of agglutinins in the serum of exposed animals was determined by a modified thread reaction. To 1 cc. of rabbit serum diluted in normal salt solution was added 0.2 cc. of an actively growing broth culture of Pneumococcus Type II. The tubes were incubated for 2 hours in the water bath at 37°C., placed in the ice box overnight, and the reactions read the next morning. Agglutinins were recorded as present in the serum only when the reactions were positive in a dilution of at least 1:10.

The presence of protective antibodies in the blood of the sprayed rabbits was determined by the ability of 0.2 cc. of serum to protect white mice against intraperitoneal injection of 0.001 cc. of Pneumococcus Type II culture, of which 0.000,001 cc. killed a normal mouse within 48 hours. The rabbit serum and culture were administered simultaneously.

Pneumococcus IIR

Of the 17 rabbits which were sprayed from 1 to 10 times with "R" forms derived from Type II pneumococcus none died from pneumococcus septicemia. Not even the 8 rabbits which were exposed 10 successive times developed any demonstrable agglutinins for Type II pneumococcus.

Neither did the sera of any of these rabbits protect mice against intraperitoneal injections of 0.001 cc. of a virulent culture of Pneumococcus Type II. As is seen from Table I the sera of these 5 animals, however, showed evidence of slight protection as demonstrated by the presence of a low degree of protective antibodies. Even the slight amount of protection afforded by these sera is not constant.

Pneumococcus Type II (SAv)

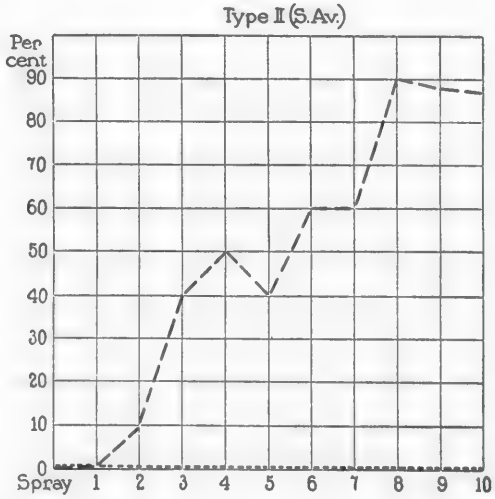
Of 11 rabbits sprayed from 1 to 10 times with the slightly virulent Pneumococcus Type II (SAv), none died from pneumococcus septi-

cemia. Not even the 8 rabbits which had completed the whole course of 10 exposures developed any demonstrable agglutinins for Type II pneumococcus.

TABLE I

First Appearance and Degree of Protection Following Repeated Exposure to Pneumococcus IIR

Following exposure.....	3	4	5	6	7	8	9	10
Rabbit 1.....	10^{-6}	—	10^{-6}	—	10^{-6}	10^{-6}	10^{-5}	
" 2.....	—	—	—	—	—	—	—	10^{-6}
" 3.....	—	—	—	10^{-5}	10^{-5}	10^{-6}	10^{-5}	10^{-4}
" 4.....	—	—	—	—	10^{-6}	10^{-6}	10^{-6}	—
" 5.....	—	—	—	—	—	—	10^{-6}	—



TEXT-FIG. 1. Comparison of presence of protective antibodies and agglutinins in rabbits following repeated inhalations of slightly virulent Type II (SAv) pneumococci.

- - - - - - - - per cent of rabbits showing agglutinins in serum following spraying.

— — — — per cent of rabbits showing protective antibodies in their serum.

Protective Antibodies

In Table II is shown the number of rabbits which were repeatedly sprayed from 1 to 10 times with Pneumococcus Type II (SAv) and the number thus exposed of which the serum subsequently protected mice against intraperitoneal injection of 0.001 cc. of a virulent culture of Type II pneumococcus. From this table it is seen that protective antibodies were demonstrable in the sera of 1 of the rabbits after the 1st spray. Following each successive spraying, the percentage of

TABLE II

Number of Rabbits Exposed to Pneumococcus II (SAv) and Number Yielding Serum Which Protected Mice against 0.001 Cc. of Pneumococcus Type II

No. of exposures	1	2	3	4	5	6	7	8	9	10
No. of rabbits	11	10	10	10	10	10	10	10	9	8
" " " whose serum protected.	—	1	4	5	4	6	6	9	8	7
Per cent of rabbits whose serum protected	—	10	40	50	40	60	60	90	88	87

TABLE III

First Appearance of Protective Antibodies in the Serum of Rabbits Exposed to Inhalation of Pneumococci Type II (SAv)

No. of spray	1	2	3	4	5	6	7	8	9	10
No. of rabbits showing protective antibodies	—	1	3	1	1	1	1	1	—	—

rabbits showing protection steadily increased, until after the 8th spraying the serum of 90 per cent of the animals conferred passive protection on mice against at least 1000 lethal doses of virulent cultures. The development of protective antibodies is graphically shown in Text-fig. 1. The exposure following which the rabbit sera first protected mice is shown in Table III. From this it is seen that with each successive spray the number of rabbits in the sera of which protective antibodies were demonstrable progressively increased. Certain rabbits showed protective antibodies only after the 7th or 8th exposure.

Pneumococcus II (Sv)

Of 50 rabbits sprayed from 1 to 10 times with the virulent strain of Type II (Sv) pneumococcus, 12 or 24 per cent died of pneumococcus septicemia after the first exposure. As the more susceptible animals succumbed early, serum was obtained only from rabbits with the greatest natural resistance, individuals which had also acquired a certain degree of immunity, as the result of repeated exposures to live pneumococci.

TABLE IV

Relation of Development of Agglutinins to the Number of Exposures to Rabbit Virulent Pneumococcus II (Sv)

No. of exposures	1	2	3	4	5	6	7	8	9	10
No. of rabbits	38	34	32	29	23	22	21	17	16	15
" " " showing agglutinins	3	3	2	2	1	2	2	2	2	2
Per cent of rabbits showing agglutinins	7	8	6	6	4	9	9	11	12	13

TABLE V

First Appearance and Titre of Agglutinins Following Repeated Inhalations of Rabbit Virulent Pneumococcus Type II (Sv)

Following exposure	1	2	3	4	5	6	7	8	9	10
Rabbit 1	1–20	—	—	—	—	—	—	—	—	—
" 2	1–10	1–10	—	1–10	—	1–10	1–10	1–20	1–40	1–40
" 3	1–10	1–50	1–200*							
" 4	—	1–20	1–20	1–50	1–40	1–10	1–10	1–10	1–10	1–10

—, no agglutinins demonstrable in serum.

* Accidental death of rabbit following 3rd exposure.

In Table IV is shown the number of rabbits which were sprayed from 1 to 10 times whose serum showed agglutinins in dilutions of at least 1 to 10. From Table IV it is seen that 3 or 7 per cent of the 38 rabbits which survived the first exposure developed demonstrable agglutinins.

Only 4 rabbits developed agglutinins. In Table V are shown the exposures after which agglutinins first appeared and the titre of agglu-

tinins following each subsequent spraying. Three rabbits showed agglutinins after the first exposure. Following the initial appearance of the agglutinins there is a tendency for the titre to rise after subsequent exposures. But in one instance the agglutinins disappeared, and in another became less marked.

Protective Antibodies

In Table VI are shown the number of rabbits that were sprayed from 1 to 10 times with rabbit virulent Pneumococcus II (Sv) and the num-

TABLE VI

Number of Rabbits Exposed to Rabbit Virulent Pneumococcus Type II (Sv) and Number That Developed Protective Antibodies in Their Serum

No. of exposures	1	2	3	4	5	6	7	8	9	10
No. of rabbits	38	34	32	29	23	22	21	17	16	15
" " " whose serum protected	6	7	7	7	4	4	4	6	6	8
Per cent of rabbits whose serum protected	10	20	21	24	17	18	29	35	37	53

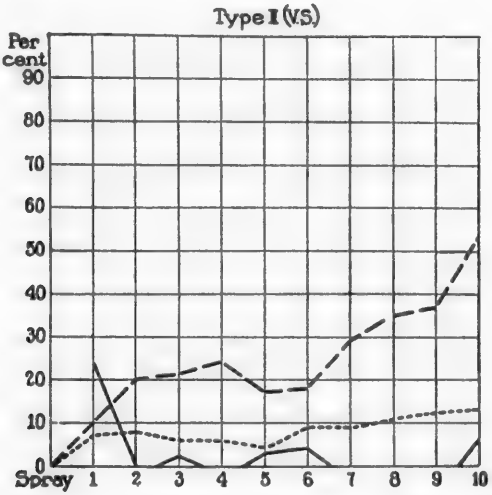
TABLE VII

First Appearance of Protective Antibodies

No. of spray	1	2	3	4	5	6	7	8	9	10
No. of rabbits first showing protective antibodies	6	1	—	1	—	—	—	1	1	3

ber thus exposed the serum of which subsequently protected mice against intraperitoneal injections of 0.001 cc. of a virulent culture of Type II (Sv) pneumococcus. From this table it is seen that protective antibodies were demonstrable in the sera of 4 or 10 per cent of the rabbits after the 1st spray. Following each successive spraying the number of rabbits yielding serum which conferred passive protection increased, until after the 10th spraying the serum of 8 or 53 per cent of the animals conferred passive protection on mice against at least 1000 lethal doses of virulent culture. The spray following which the sera first protected mice is shown in Table VII.

From Table VII it is seen that after the 1st exposure the sera of 6 rabbits showed protective antibodies. After successive sprays an occasional rabbit would first show protective antibodies. Protective substances were first demonstrated in the sera of 3 rabbits only after the 10th exposure.



TEXT-FIG. 2. Comparison of mortality and presence of protective antibodies and agglutinins in rabbits following repeated inhalations of virulent Type II (Sv) pneumococci.

———— per cent of rabbits dying with pneumococcus septicemia following successive sprayings.

- - - - - - - - per cent of rabbits showing agglutinins in serum following spraying.

— — — — per cent of rabbits showing protective antibodies in their serum.

Correlation of Agglutinins and Protective Antibodies

It is difficult to compare the relative titre of the sera at any one time because of the difference in the standards used. Whereas agglutinins were recorded as positive if present in serum concentration of 1:10, protective antibodies were noted only if they were present in concentrations sufficient to protect mice against a 0.001 cc. of virulent pneumococcus culture. If smaller doses of pneumococcus had been used, the

presence of protective antibodies would undoubtedly have been detected earlier and the incidence of their occurrence would have been more frequent. However, when these antibodies were demonstrable under the conditions of this experiment, their presence was evidence of the capacity of the serum to confer a high degree of passive immunity on mice.

In 3 instances both agglutinins and protective antibodies occurred after the same spray; in another rabbit, yielding serum that contained agglutinins, protective antibodies appeared after the next exposure. In 9 instances protective antibodies occurred without demonstrable agglutinins in the serum and the sera of 7 rabbits, even after 10 exposures, contained no agglutinins or protective antibodies even sufficient to protect mice against 0.000,001 of a virulent Type II pneumococcus.

The relative occurrence of agglutinins and of protective antibodies in the serums of rabbits following inhalations of rabbit virulent strain of Type II (Sv) pneumococcus is graphically shown in Text-fig. 2. In this figure the mortality curve of rabbits dying from pneumococcus septicemia is also given. It is seen that after the 1st exposure 24 per cent of the rabbits died from pneumococcus septicemia. An occasional rabbit died following subsequent exposures. The sera of the rabbits dying after the 3rd, 5th and 6th exposures showed neither agglutinins nor protective antibodies. The serum of the rabbit dying after the 10th spray, however, protected mice against 0.01 cc. of pneumococcus culture. It is unfortunate that it is not known if the pneumococcus recovered from this rabbit was the original type specific organism used for spraying or a degenerated "R" form. The number of rabbits that developed agglutinins was never great, nor was the titre high. The proportion of rabbits showing agglutinins rose only slightly following successive exposures. On the other hand, there was a distinct tendency for the proportion of rabbits to develop protective antibodies with each successive spray. Following the 10th exposure 53 per cent of the total number of survivals showed the presence of these immune substances in their serum.

DISCUSSION

Although too few rabbits were used to give as definite results as after spraying with Type I pneumococcus, some definite results are

indicated. It appears that the immunological reactions of rabbits following inhalations of the same type of pneumococci vary in direct proportion to the virulence of the organism. In the case of exposure to a non-virulent degenerated "R" strain derived from Type II pneumococcus no type specific antibody response was elicited. If rabbits are allowed to inhale slightly virulent Type II (SAv) pneumococcus, none succumb to infection, although a certain number develop a pneumococcus septicemia. As the result of successive spraying a large proportion of the animals developed protective antibodies in their serum but no agglutinins. Following the initial exposure to a rabbit-virulent Pneumococcus Type II (Sv) the greatest number of rabbits succumbed to a fatal septicemia. Certain rabbits developed a high degree of immunity as evidenced by the presence of agglutinin and protective antibodies in their serum. Why a larger proportion of rabbits exposed to the slightly virulent Pneumococcus Type II (SAv) developed protective antibodies than those sprayed with the virulent Pneumococcus Type II (Sv) is difficult to explain. The reaction of the rabbits to inhalations of the virulent Type II (Sv) culture parallels the results obtained when rabbits were sprayed with a rabbit-virulent Type I pneumococcus.

From the foregoing experiments it is certain that following inhalation of living virulent pneumococci of Type I or II rabbits develop a high degree of immunity. This immunity is probably induced by a few organisms penetrating the respiratory epithelium and entering into the body tissues. It has already been shown that rabbits may recover from a transient pneumococcus septicemia following inhalation of pneumococci. The great variations both in the time of first appearance and in the final titre of immune bodies are difficult to explain.

Among the factors which cannot be experimentally controlled are: first, the number of organisms which come to lodge within the respiratory tract following exposure to a bacterial spray; second, the number which are able to invade the tissues after implantation; and third, the final disposition of these bacteria in the animal body. In certain instances the multiplication of the invading organisms goes on unchecked until the death of the animal. In others a transient carrier state may occur with subsequent immunity responses. It is certainly significant that whereas the curve of incidence of protective antibodies

steadily increases, the percentage of rabbits showing agglutinins does not materially change after the 5th spraying.

CONCLUSIONS

1. Following repeated inhalations of the degenerated non-virulent "R" forms of Type II pneumococcus, no type specific antibodies can be demonstrated in the serum of rabbits.

2. Following repeated inhalations of slightly virulent Type II (SAv) pneumococci, only protective antibodies can be demonstrated in the serum of rabbits.

3. Following repeated inhalations of virulent Type II (Sv) pneumococci, agglutinins and protective antibodies can be demonstrated in the serum of rabbits.

4. Following repeated exposures of rabbits to inhalation of pneumococci, the type specific response, evidenced by type specific protective antibodies and agglutinins, varies in direct proportion to the virulence of the culture used.

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A TYPE SPECIFIC SUBSTANCE DISTINCT FROM THE SPECIFIC CARBOHYDRATE IN PNEUMOCOCCUS TYPE I

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(Received for publication, May 12, 1930)

The facts first determined by Avery and Heidelberger (1) and Zinsser and Tamiya (2) concerning the species specific nucleoprotein fraction of the pneumococcus as well as the type specific carbohydrate substance are at present so widely known and generally recognized that their recapitulation here would be superfluous. Aside from these fractions, other workers have described antigenic substances obtained by various chemical manipulations. Perlzweig and Steffen (3) in 1923 reported the finding of a material derived from the pneumococcus which was capable of active immunization upon injection into rabbits but which survived digestion with trypsin, was non-lipoidal, and gave Millon, xanthroproteic and ninhydrin reactions. Perlzweig and Keefer (4) in 1925 described an actively immunizing substance of a protein nature isolated from broth culture filtrates of Pneumococcus Type I by ultrafiltration, precipitation at a definite hydrogen ion concentration and the separation of a soluble picrate fraction. Jungeblut (5) has described an alcohol soluble substance which under certain conditions reacts specifically by precipitation with type sera. The latest studies of Julianelle (6) on skin and conjunctival hypersensitiveness in rabbits treated with products of the pneumococcus suggest the presence of a third non-type specific substance in that organism.

In this paper evidence will be presented which points to the presence of a substance other than the specific carbohydrate in the autolytic products of Pneumococci Type I which reacts specifically with immune serum as determined either by the precipitin reaction, or, *in vivo*, by the anaphylactic behavior of guinea pigs appropriately sensitized.

Primary Observation

The possibility of the existence of a substance quite distinct from the type specific carbohydrate was suggested by the behavior of the

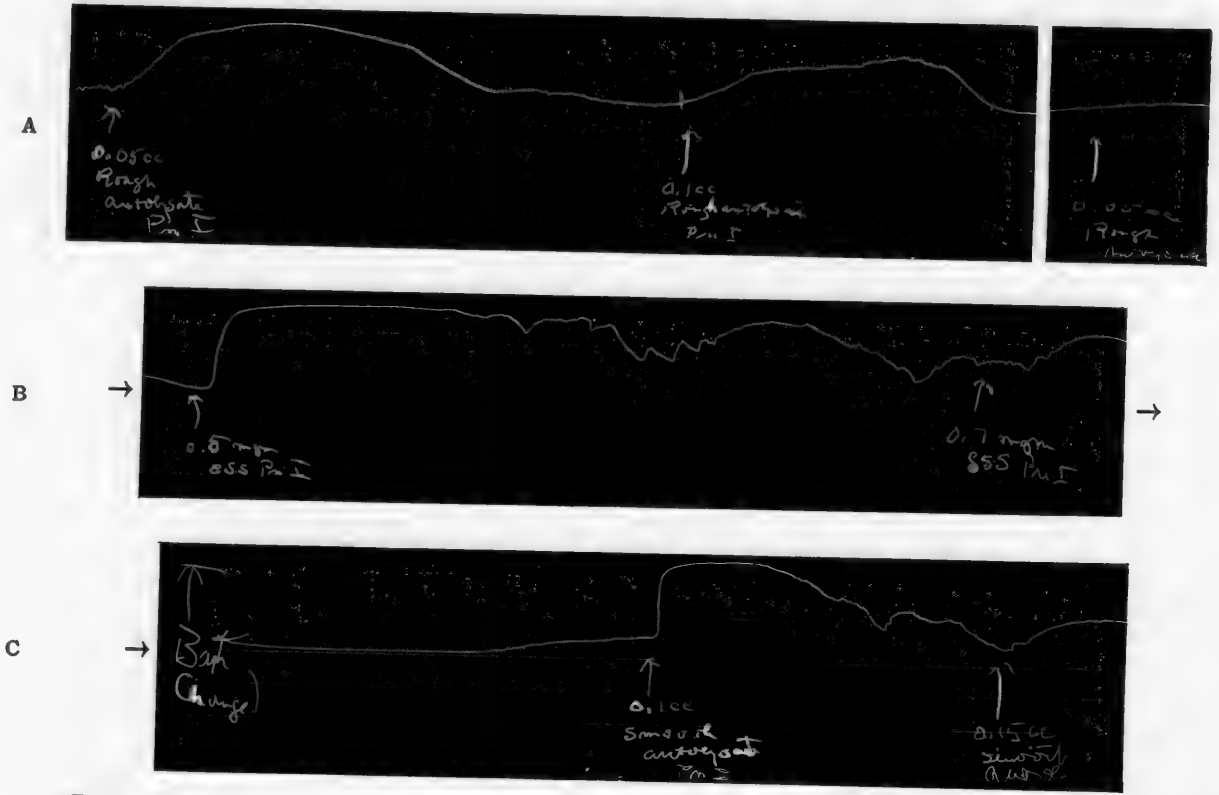

FIG. 1. The sections above represent the continuous tracing of one uterine horn of a guinea pig sensitized 24 hours before the experiment was performed by the intraperitoneal injection of 1 cc. of antipneumococcus Type I rabbit serum. In (A) is shown the effect of the autolysates from a "rough" strain of Pneumococcus Type I, in (B) that of Type I specific soluble substance, and in (C) the reaction caused by the autolysate from a "smooth" strain of Pneumococcus Type I. The volume of Ringer's solution in the bath was 25 cc.

uterine horns of guinea pigs which had been passively sensitized with antipneumococcus Type I rabbit serum. It was found that the uterine strip when treated with the following materials in the order named responded typically to each in turn:

1. Autolysate from a "rough" strain of pneumococcus.
2. The homologous specific carbohydrate purified according to the method of Heidelberger and Avery.
3. The autolysate obtained from a "smooth" strain of Pneumococcus Type I. (A representative tracing is reproduced in Fig. 1).

Desensitization of the uterus to each substance was demonstrated before the addition of a different material. It was also shown that in the quantities used, these materials had no comparable effect upon the uterus of a normal guinea pig. This result indicated that the autolysate obtained from the "smooth" or virulent Pneumococci Type I contained a substance capable of causing a typical contraction of the uterine muscle after it had been shown to be completely unresponsive to the nucleoprotein fraction (as represented by R autolysate) and to the purified specific carbohydrate. This substance has provisionally been designated the "A" substance.

The remainder of this communication consists in a description of the methods and results employed in the confirmation of the significance of this primary observation.

EXPERIMENTAL

In every instance the autolysates were produced by centrifuging, in large bottles, cultures of pneumococci grown for 24 hours in 0.1 per cent dextrose hormone broth. The sediment from about 250 cc. broth culture was taken up in 1 cc. of saline. The suspensions were collected and adjusted to contain 0.5 per cent phenol. The mixture was allowed to stand for about 72 hours at 37°C. and then kept in the ice box. The undissolved débris was centrifuged off before the clear supernatant autolysate was used in any test.

The antipneumococcus Type I rabbit serum hereafter to be designated as "normal" antipneumococcus rabbit serum was obtained in the usual manner by treating rabbits intravenously with cultures of Pneumococci Type I grown in broth containing 1 per cent rabbit's blood and killed by the addition of 0.3 per cent formalin.

The antipneumococcus Type I rabbit serum hereafter to be designated as anti-A Pneumococcus Type I rabbit serum was produced by the following procedure:

Heavy suspensions of pneumococci obtained by centrifuging 0.1 per cent dextrose hormone broth cultures, and taking up the sediment of 1 cc. saline, were treated with sufficient formalin in 10 per cent solution to give a final concentration of 0.2 per cent. After 24 to 48 hours at 37°C. tests for sterility were made. Rabbits were treated intravenously at suitable intervals with rather large amounts of the formalinized suspension.

It will be particularly noted that when heavy suspensions such as those employed are treated in the manner described, what may be spoken of as a "partial" autolysis appears to take place, *i.e.*, the organisms become Gram negative and seem to become somewhat smaller. In the case of two of the sera (228 and 230), doses of formalinized broth culture of pneumococci were given to the rabbits subsequently to the large amounts of partially autolyzed organisms. The details concerning the manner in which anti-A pneumococcus rabbit Sera 228 and 230 were prepared are given in Table I. It must be admitted that the immunological principles responsible for the production of sera which do not develop the S antibody but which do contain an antibody against the A substance are entirely unknown. Accordingly the presentation of a reliable technique for obtaining such sera cannot be given. Subsequently it will be shown that "normal" antipneumococcus Type I sera contain the A antibody which remains in the serum after the antibody reacting with the specific carbohydrate has been removed by precipitation. The most convenient and readily available method for obtaining an antiserum against the A substance depends upon this fact.

The nucleoprotein was obtained by precipitation with dilute acetic acid solution derived by dissolving pneumococci with bile according to the method of Avery and Heidelberger (7).

The specific carbohydrate was produced from pneumococci grown on 0.1 per cent dextrose hormone broth according to the methods described by Heidelberger and his associates. Owing, probably, to a slight amount of $BaSO_4$ in the Type I carbohydrate, the nitrogen present was equivalent to only 3.7 per cent.

Demonstration of the Presence of the A Substance in Antipneumococcus Type I Sera by Means of the Precipitin Test.—It was found that antipneumococcus rabbit sera produced by injecting the so-called "partially" autolyzed organisms gave no precipitation upon the addition of the specific carbohydrate. On the other hand, when the homologous autolysate was added, an abundant flocculent precipitate formed immediately. That this precipitate was only in a minor proportion due to the presence in the serum of a nucleoprotein antibody is shown by the results recorded in Tables II and III, which also demonstrate the absence of an antibody capable of reacting with the specific soluble substance.

A further indication that the species specific nucleoprotein is not responsible for the bulk of the precipitate obtained by adding the

TABLE I

Immunization of Rabbits Producing Anti-A Pneumococcus Serum

Serum	Days after first injection of vaccine							
	0	3	6	18	21	23	29	36
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
228	0.5 F	0.5 F	0.5 F	0.5 BC	1.0 BC	2.0 BC	5.0 BC	1.0 HB
230	1.0 F	1.0 F	0.5 F	0.5 BC	1.0 BC	2.0 BC	5.0 BC	—

F = Centrifugate from 250 cc. 0.1 per cent dextrose hormone broth culture Pneumococcus Type I taken up in 1 cc. saline. 0.2 per cent formalin. Culture grown for 16 hours.

BC = 16 hour hormone broth culture containing 1 per cent rabbit blood. 0.2 per cent formalin.

HB = 1/10 dilution of vaccine prepared by washing growth from horse blood agar pie plate in 5 cc. 0.2 per cent formalin solution in saline.

All vaccines were injected intravenously.

TABLE II

Titration of Anti-A Pneumococcus I Rabbit Sera with the Specific Soluble Substance and the Homologous Autolysate

Antigen		1/1	1/10	1/100	1/1,000	1/10,000	1/100,000	1/1,000,000	1/2,000,000	1/4,000,000
S S S Pn. I	Serum 228	Not done	Not done	—	—	—	—	—	—	—
	Serum 230	Not done	Not done	—	—	—	—	—	—	—
Autolysate Pn. I (smooth)	Serum 228	++++	++++	+++±	+++	±	—	—	—	—
	Serum 230	+++±	+++	++±	++	±	—	—	—	—

++++ = very heavy precipitate falling to bottom of tube. +++ = precipitate falling to bottom of tube. ++ = heavy ring—stays up. + = definite ring. ± = ring just discernible.

The ring test technique was employed in this and the following precipitin tests in which the antigen was diluted. Readings taken after 2 hours at room temperature.

autolysate of Pneumococcus Type I to this serum is offered by the experiment recorded in Table IV. Type I autolysate was treated with 10 per cent acetic acid in the cold until test of a portion of the fluid indicated a pH of about 4. After standing over night in the cold, the acid-precipitable material was removed by centrifugation. The supernatant fluid was tested with acetic acid. Any further precipitate which formed after 2 hours in the ice box was removed and the supernatant fluid neutralized with sodium hydroxide. When this material was tested against an anti-"rough" Pneumococcus I rabbit serum

TABLE III

Titration of an Anti-A Pneumococcus Rabbit Serum, Employing Various Pneumococcus Autolysates as Antigens

Antigen	Dilution of serum						
	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Pn. I autolysate (smooth)	++++	+++±	++±	++	+	±	−
Pn. II autolysate (smooth)	−	−	−	−	−	−	−
Pn. II autolysate (rough)	±	−	−	−	−	−	−
Pn. III autolysate (smooth)	±	−	−	−	−	−	−
Pn. I S S S	−	−	−	−	−	−	−

In this experiment the antiserum was diluted while the amount of antigen was kept constant. 1 drop of undiluted autolysate was added to 0.25 cc. of the serum dilution. 1 drop of the specific soluble substance diluted 1/10,000 was added to the same volume of diluted serum. Readings taken after 2 hours at room temperature.

which had a precipitin titre of 1:3200 (dilution of antigen) against the chemically isolated nucleoprotein, it produced no precipitate in the dilution employed. When, however, it was titrated with an anti-A serum, containing no precipitating antibody for the specific soluble substance, it showed only a slight diminution in precipitating capacity as compared with the untreated autolysate against the same serum. This diminution may in large part be accounted for by the loss of the nucleoprotein.

It may also be mentioned in this connection, although a discussion of the chemical aspects of the A substance will be temporarily deferred,

that it is possible to obtain by acid and alcohol precipitation a material which fails to give any precipitin reaction with the anti-"rough" pneumococcus serum mentioned above, but which reacts strongly with anti-A serum. This fact, taken with the evidence already presented, together with that which is to follow, makes it quite clear that the nucleoprotein fraction cannot be identified with the A substance, while the complete failure of the purified specific soluble substance to produce precipitation in these sera conclusively indicates that this substance is not the precipitating antigen concerned in the reactions.

In the light of the foregoing facts and the evidence afforded by the

TABLE IV

Titration of the A Substance before and after Precipitation with Acetic Acid of the Nucleoprotein from the Homologous Autolysate

Antigen		Dilution of antigen					
		1/8	1/32	1/128	1/512	1/2048	1/8192
Autolysate Pn. I (smooth)	Anti-R Pn. serum	++	+	−	−	−	−
	Anti-A Pn. I serum	+++±	+++	++±	++	+	−
Supernatant from acetic acid precipitation of autolysate Pn. I (smooth)	Anti-R Pn. serum	−	−	−	−	−	−
	Anti-A Pn. I serum	+++	++±	++	+±	+	−

Readings taken after 2 hours at room temperature.

isolated uterus of a guinea pig sensitized with antipneumococcus Type I rabbit serum prepared in the customary manner, it became of interest to determine, if possible, by the method of specific precipitation whether or not "normal" antipneumococcus rabbit serum contains the A antibody.

A technique was devised* which enabled us to demonstrate the presence of this antibody in serum which originally contained a large amount of antibody reacting

* Dr. Hugh K. Ward in this laboratory devised this technique for eliminating a precipitating antibody from an antiserum.

with the specific carbohydrate. By titration the quantity of the purified specific soluble substance was found which caused the optimum precipitate in a "normal" antipneumococcus Type I rabbit serum. The calculated amount of carbohydrate was then added to any desired volume of the antiserum and, after 2 hours at 37°C., the precipitate was removed by centrifugation. A portion of the supernatant fluid was again titrated against varying quantities of the specific soluble substance and the optimum precipitate recorded. The calculated amount of carbohydrate causing this precipitate was added to the bulk of the supernatant fluid and after 2 hours at 37°C. the precipitate was removed. The supernatant from this precipitation was then tested with varying dilutions of the carbohydrate.

Usually no visible precipitation occurred with any dilution of the specific soluble substance employed in the tests. When tested, however, with the homologous autolysate, an abundant flocculent precipitate was observed. These results, together with those obtained with the "rough" Pneumococcus I autolysate are summarized in Table V. The autolysates of heterologous types freed from the acid precipitable nucleoprotein failed to give definite precipitates with these sera. In addition data are included which were recorded in an experiment with a therapeutic Type I antipneumococcus horse serum treated in an identical manner which was produced at the Massachusetts State Antitoxin Laboratory.

The precipitin tests with "normal" Pneumococcus Type I horse and rabbit sera lend strong support to the evidence presented by the conduct of the isolated uterus for the presence of a type specific substance in the homologous autolysate which is distinct from the specific carbohydrate.

Passive Anaphylaxis in Guinea Pigs Sensitized with Anti-A Pneumococcus Rabbit Serum.—Further proof of the existence of the A substance was sought in experiments which employed the systemic anaphylactic reaction in guinea pigs passively sensitized with anti-A rabbit serum or with a "normal" antipneumococcus rabbit serum from which the S antibody had been removed by means of the procedure described above.

It was found that guinea pigs injected intraperitoneally with 1 to 2 cc. of both anti-A rabbit sera used in the precipitin tests failed to develop symptoms of anaphylaxis upon intravenous injection of varying quantities of the purified specific carbohydrate. Nor was the antibody against the nucleoprotein or "rough" autolysate present in

sufficient concentration in these sera to confer upon guinea pigs anaphylactic sensitivity to these substances. Again, no anaphylaxis developed in animals treated with such sera when autolysates derived from either Type II or Type III virulent strains of pneumococci were introduced intravenously. The Type I anti-A sera did, however,

TABLE V

Demonstration of the A Precipitating Antibody in "Normal" Antipneumococcus Type I Sera from Which the Antibody Reacting with the Specific Substance Had Been Removed by Precipitation

Serum	Antigen	Dilution of antigen					
		1/2	1/8	1/32	1/128	1/512	1/2048
Antipneumococcus I rabbit serum	S I A	+++±	+++	++±	++	++	+±
	S II A	+	−	−	−	−	−
	S III A	+±	±	−	−	−	−
	R I A	+±	−	−	−	−	−
Antipneumococcus I horse serum	S I A (ppt.)	+++±	+++	++±	+±	+	±
	S III A (ppt.)	−	−	−	−	−	−
	R I A	++	++	+	±	−	−

S I A = Pneumococcus I autolysate (smooth).

R I A = Pneumococcus I autolysate (rough).

S II A = Pneumococcus II autolysate (smooth).

S III A = Pneumococcus III autolysate (smooth).

ppt. = Autolysate treated with dilute acetic acid in the cold to remove nucleoprotein. This was done in the case of the horse antiserum because it contained a large amount of "P" antibody.

Both horse and rabbit antisera after removal of the S S S antibody showed no precipitation with the specific soluble substance in dilutions of the latter ranging from 1/1000 to 1/4,000,000.

Ring tests—readings after 2 hours at room temperature.

regularly confer upon guinea pigs a very high degree of anaphylactic sensitivity to the autolysate derived from Type I pneumococcus. The "normal" antipneumococcus Type I rabbit serum from which the S antibody had been removed also rendered guinea pigs anaphylactically hypersensitive to the A substance in the homologous autolysate. Before the removal of the S antibody by precipitation *in vitro*, this serum

sensitized guinea pigs anaphylactically to the type specific soluble substance. After the S antibody had been eliminated, animals sensitized with this serum failed to react anaphylactically upon the injection of the carbohydrate, but did respond typically upon being injected with the autolysate from Type I virulent organisms. In addition, they showed no symptoms upon intravenous treatment with autolysate derived from a "rough" strain of Type I or from autolysates of smooth strains of Types II and III. The results of these experiments are summarized in Table VI.

Although not relevant to this subject, it is of interest to note that in these experiments with a rabbit serum which at first contained two different antibodies capable of anaphylactic sensitization, but which was deprived of one of these antibodies by precipitation with the specific antigen, the complete dependence of the anaphylactogenic antibody upon the precipitating antibody is made very clear. That these two antibodies are distinct immunological entities is still held by certain workers. There is, of course, no final proof of their identity in these experiments—a proof that can only be obtained when a chemical isolation of an antibody is accomplished. Yet the fact that the sensitizing antibody may be completely removed *in vitro* by precipitation with the specific antigen from a serum in which a second sensitizing antibody remains undisturbed is additional evidence for regarding them as one.

These anaphylaxis experiments show that in the sera of rabbits which have received injections of formalinized pneumococci an antibody develops which reacts specifically with an antigen found in the autolytic products of Type I pneumococcus. Similar materials obtained from the other two types of organism, as well as from "rough" strains of the homologous and heterologous types cause no anaphylactic symptoms. The chemically prepared nucleoprotein is likewise incapable of eliciting anaphylaxis. That the antigen in the Type I autolysate responsible for the anaphylactic symptoms is not the specific carbohydrate appears to be definitely shown not only by the failure of the latter material when pure to produce shock in guinea pigs sensitized with anti-A rabbit sera, but also by the experiments with the rabbit serum from which the S antibody originally capable of sensitizing had been removed, leaving unimpaired the serum's power to sensitize to the A substance.

TABLE VI

Anaphylaxis Experiments with Guinea Pigs Passively Sensitized with Anti-A Rabbit Sera

Guinea pig	Amount serum injected I. P.	S S S	S I A	R I A	S II A	R II A	S III A	N P	Results
Anti-A Pneumococcus I Rabbit Serum 228									
1	2 cc.	2 mg.	—	—	—	—	—	—	No symptoms
2	2 cc.	1 mg.	0.4 cc.	—	—	—	—	—	S S S = no symptoms S I A = death in 3 minutes. Autopsy typical
3	1 cc.	1 mg.	0.4 cc.	—	—	—	—	—	S S S = no symptoms S I A = death in 3 minutes. Autopsy typical
4	1 cc.	—	0.2 cc.	—	—	0.2 cc.	—	—	R II A = no symptoms S I A = death in 4 minutes. Autopsy typical
5	1 cc.	—	0.2 cc.	—	—	0.2 cc.	—	—	R II A = no symptoms S I A = death in 4 minutes. Autopsy typical
6	1 cc.	—	0.2 cc.	—	0.2 cc.	—	—	—	S II A = no symptoms S I A = death in 3 minutes. Autopsy typical
7	1 cc.	—	0.1 cc.	0.2 cc.	—	—	—	—	R I A = no symptoms S I A = death in 3 minutes. Autopsy typical
8	1 cc.	1 mg.	0.2 cc.	0.2 cc.	—	—	—	—	S S S = no symptoms R I A = no symptoms S I A = death in 3 minutes. Autopsy typical
9	1 cc.	2 mg.	0.05 cc.	—	—	—	0.2 cc.	—	S S S = no symptoms S III A = no symptoms S I A = death in 4 minutes. Autopsy typical

TABLE VI—*Continued*

Guinea pig	Amount serum injected I. P.	S S S	S I A	R I A	S II A	R II A	S III A	N P	Results
					Anti-A Pneumococcus I Rabbit Serum 230				
10	1 cc.	1 mg.	0.1 cc.	0.2 cc.	—	—	—	—	S S S = no symptoms R I A = no symptoms S I A = death in 3 minutes. Autopsy typical
11	1 cc.	1 mg.	0.1 cc.	—	0.2 cc.	—	—	—	S S S = no symptoms S II A = no symptoms S I A = death in 3 minutes. Autopsy typical
12	1 cc.	1 mg.	0.1 cc.	—	—	—	0.2 cc.	—	S S S = no symptoms S III A = no definite symptoms* S I A = death in 5 minutes. Autopsy typical
13	1 cc.	—	0.2 cc.	—	—	—	—	3 mg.	N P = no symptoms S I A = death in 3 minutes. Autopsy typical
					Anti-Pneumococcus I Rabbit Serum without S S S Antibody				
14	1 cc.	2 mg.	0.2 cc.	0.2 cc.	—	—	—	—	S S S = no symptoms R I A = no symptoms S I A = death in 3 minutes. Autopsy typical
15	1 cc.	1 mg.	0.1 cc.	—	—	—	—	—	S S S = no symptoms S III A = no symptoms S I A = death in 3 minutes. Autopsy typical
16	1 cc.	1 mg.	0.1 cc.	—	0.2 cc.	—	—	—	S S S = no symptoms S II A = no symptoms S I A = death in 6 minutes. Autopsy typical

S S S = Pneumococcus I specific soluble substance.
S I A = Pneumococcus I (smooth) autolysate.
R I A = Pneumococcus I (rough) autolysate.
S II A = Pneumococcus II (smooth) autolysate.
R II A = Pneumococcus II (rough) autolysate.
S III A = Pneumococcus III (smooth) autolysate.
N P = Pneumococcus I nucleoprotein.

The sensitizing serum was injected intraperitoneally 24 hours before the test substances were administered intravenously (ear vein). An interval of about 10 minutes was allowed to elapse between the intravenous injection of the various test materials. In every case the specific soluble substance was injected first. This was followed by the non-specific autolysate or nucleoprotein. The Pneumococcus I (smooth) autolysate was injected last. The Pneumococcus I (smooth) autolysate in the amounts used in these experiments produced no symptoms in normal guinea pigs.

* Guinea pig 12 after the injection of Type III autolysate showed weakness and rapid respiration. Recovery was immediate. These symptoms were not interpreted as being anaphylactic.

The autolysate prepared from a different strain of virulent Type I pneumococcus was tested both by the methods of precipitation and of anaphylaxis for the A substance, which was found to be present.

TABLE VII

Effect of Heat on the A Substance

		Dilution of antigen					
		1/8	1/32	1/128	1/512	1/2048	1/8192
Anti-A Pneumococcus Type I rabbit serum 228	Untreated phenolized S I A	++++±	+++	++±	++	+	+
	Supernatant after treatment with HAc	+++	++±	++	+±	+	−
	HAc supernatant boiled at pH 9	±	−	−	−	−	−
	HAc supernatant boiled at pH 4	+++	++±	++	+±	+	−
	HAc supernatant autoclaved at pH 9	−	−	−	−	−	−
	HAc supernatant autoclaved at pH 4	+*	±*	−	−	−	−
Anti-R Pneumococcus Type I rabbit serum	Untreated phenolized S I A	++	+	−	−	−	−
	Supernatant after treatment with HAc	−	−	−	−	−	−
	HAc supernatant boiled at pH 9	−	−	−	−	−	−
	HAc supernatant boiled at pH 4	−	−	−	−	−	−
	HAc supernatent autoclaved at pH 9	−	−	−	−	−	−
	HAc supernatant autoclaved at pH 4	±*	−	−	−	−	−

* These reactions may probably be interpreted as non-specific, since the HAc supernatant did not give this reaction with anti-R serum before autoclaving at pH 4.

S I A = Pneumococcus Type I (smooth) autolysate.

HAc = Acetic acid.

Solutions boiled for ½ hour.

Solutions autoclaved for 1 hour at 15 pounds pressure.

The ring test was employed in this experiment. Readings after 2 hours at room temperature.

Physical and Chemical Characteristics of the A Substance

Effect of Heat.—A characteristic of the A substance which sharply distinguishes it from the soluble specific substance is the destructive

effect of heat upon it when in weakly alkaline solution. If boiled for ½ hour at pH 9, its effectiveness as a precipitating antigen is reduced at least one thousand fold, while the specific carbohydrate remains unchanged. On the other hand, when the reaction is adjusted to pH 4 with 10 per cent acetic acid, a solution containing the A substance may be boiled for the same period of time without losing its activity. In both acid and alkaline solution, autoclaving for 1 hour at 15 pounds pressure practically destroys its effectiveness. In the experimental results recorded in Table VII, the evidence for these statements is presented. To eliminate in so far as is possible the nucleoprotein fraction, the autolysate used in these experiments was

TABLE VIII

Effect of Peptic Digestion on A Substance

Serum		Dilution of the antigen				
		1/1	1/8	1/32	1/128	1/152
Anti-A Pneumococcus I rabbit serum 228	After digestion	+++	++	+±	+	−
	Control (undigested)	+++	++	+±	+	−
Anti-R Pneumococcus rabbit serum	After digestion	−	−	−	−	−
	Control (undigested)	++	±	−	−	−

Readings taken after 2 hours at room temperature.

first treated with acetic acid and the resulting precipitate discarded before the supernatant fluid containing the A substance was either boiled or autoclaved.

Effect of Peptic and Tryptic Digestion.—Digestion for 72 hours in solution containing 1 per cent pepsin at 37.5°C. does not decrease the precipitating power of the A substance. To 1 cc. of Pneumococcus I autolysate, 1 cc. of 2 per cent solution of pepsin in 0.1 N hydrochloric acid was added. The mixture was then placed in a "celofane" dialyzing bag and immersed in a solution of 0.1 N hydrochloric acid. The liquid outside the bag was changed twice each day. As a control a similar mixture of autolysate and boiled pepsin solution was dialyzed under the same conditions. The enzyme in a concentration of 1 per

cent readily digested coagulated egg white (Mett tube). Its activity was also indicated by the reduction in the precipitating strength of the digest when added to an anti-R pneumococcus rabbit serum. This loss of precipitating capacity is, no doubt, to be attributed to the digestion of the nucleoprotein by the enzyme. In Table VIII are presented the results of an experiment to determine the effect of pepsin on the A substance.

Because of the fact that in weakly alkaline solution the activity of the A substance seems to be markedly impaired by prolonged exposure to a temperature of 37.5°C., it was not possible to determine so clearly the effect of tryptic digestion on it. It may be stated, however, that a solution of the A substance exposed to the action of this enzyme loses no more of its capacity to react with an A antiserum than does a control solution to which no trypsin has been added but which has been subjected to identical conditions.

Chemistry of the A Substance.—Until the completion of a study now being purused which is concerned with the isolation and properties of this substance, little can be definitely stated in respect to its chemistry. In many of its reactions it resembles the specific soluble substance. Precipitated by the same volume of alcohol, traces of it are to be found in that material until very near the end of the purifying process. It is precipitated by phosphotungstic acid and by silver nitrate. It is is not precipitated either by acetic or hydrochloric acid. Until isolated in a state at least approaching purity, the tests for protein have, of course, no meaning.

DISCUSSION

In the preceding sections experimental evidence has been presented which leads to the conclusion that in the autolytic products of Pneumococcus Type I there is to be found a substance of unknown chemical composition reacting specifically both in precipitin and anaphylactic reactions with sera produced by injecting rabbits with formalinized pneumococci. This substance is distinguished from the specific carbohydrate not only by its specific precipitating and anaphylactic action with such sera, but also by its instability when boiled in weakly alkaline solution. Although this last characteristic is analogous to the conduct of certain proteins which undergo racemization under similar

conditions, the fact that pepsin and, less clearly, trypsin do not digest the A substance renders exceedingly doubtful its ultimate identification as a protein. At present the chemical aspects of the problem are being investigated.

The hypothesis of Landsteiner concerning the "haptene" or partial antigen so conclusively confirmed experimentally in the case of the lipoidal antigen of Forssmann and the bacterial carbohydrates, must inevitably be considered in relation to any substance which reacts *in vitro* with an antiserum. Since the chemical isolation of the A substance has not yet been accomplished, it is obviously impossible to arrive at any definite conception in respect to its function as a complete or partial antigen. *A priori*, however, the facts mentioned above, which suggest a substance of non-protein nature, also make it unlikely that the A antigen *per se* possesses the capacity to stimulate the production of antibody when injected into an animal.

The work of Reimann (9) and others has shown that immunization with the autolytic products of the pneumococcus produces an antibody reacting only with the nucleoprotein fraction. Although their results do not definitely preclude the possibility that an A antibody was also present in small concentration, they render it improbable. Had an A antibody been present in a serum along with the nucleoprotein antibody, that serum would have exhibited a higher precipitin titre against the homologous autolysate than against heterologous autolysates. Actually, it was found that in the sera of an animal immunized with the autolysate from any pneumococcus or with the nucleoprotein fraction the precipitin titre was the same whether the homologous or heterologous autolysate was employed as antigen.

We have not attempted an extensive series of experiments to determine whether or not an antibody against the A substance developed in the sera of rabbits immunized with autolysate derived from Pneumococcus Type I. In the sera of three animals treated in this way, however, no antibody against the A substance was demonstrated. Thus, until further data are available, the preponderance of evidence is in favor of regarding the A substance as belonging to the order of haptenes.

Whether or not materials analogous to the A substance in Pneumo-

coccus Type I exist in the autolytic products of Pneumococci Type II and Type III has not yet been definitely shown.

CONCLUSIONS

1. Evidence has been presented for the existence of a substance distinct from the specific carbohydrate in the autolytic products of Pneumococcus Type I.

2. The substance reacts specifically by precipitating homologous antiserum which either occurs naturally without antibody against the specific carbohydrate or has been deprived of that antibody artificially.

3. In guinea pigs passively sensitized with such antisera the homologous autolysate containing the substance alone produces typical lethal anaphylactic shock.

4. In weakly alkaline solution the substance is destroyed by boiling. In weakly acid solution it resists a temperature of 100°C. for at least ½ hour. Autoclaving for 1 hour at 15 pounds pressure in either acid or alkaline solution destroys its activity as precipitinogen.

5. The substance is resistant to peptic digestion.

6. The chemical nature and the possible identification of the substance as a haptene have been discussed.

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THE INFLUENCE OF ULTRA-VIOLET RADIATION ON THE WEIGHT OF ADULT RABBITS, NORMAL AND SYPHILITIC

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(Received for publication, May 14, 1930)

During the course of an experiment previously reported (1), it was noted that rabbits exposed to ultra-violet radiation under certain conditions did not appear to be in as good physical condition as animals living in an environment from which all light was excluded and were more susceptible to intercurrent infections. With these facts in mind, the experiment was repeated with a view to determining the influence of ultra-violet irradiation on body weight, the chemical composition of the blood, and susceptibility to intercurrent infections of normal and syphilitic animals and on the reaction to syphilitic infection. The present paper will be limited to a report of the results obtained for body weight and susceptibility to intercurrent infections.

Material and Methods

In the experiment to be reported, 60 male rabbits between 6 and 8 months of age were used. The animals were divided into 6 comparable groups of 10 each, and from the beginning of the experiment on November 27, 1928, until June 15, 1929, these animals were kept in a room from which all light was excluded. They were caged separately, and fed the routine diet of hay, oats, and cabbage. The temperature of the dark room was satisfactorily maintained at 70° to 75°F. and the humidity varied with that of the outside air. The animals were weighed at weekly intervals.

Beginning December 5, 1928, 30 animals, listed as Groups II, IV, and VI were exposed for an hour each day to the unfiltered radiations of a quartz-mercury arc lamp (80 volts, 4.2 amperes) at a distance of 4.5 meters. The doors of the cages were open to permit free access of light but no depilatory measures were used and at no time throughout the experiment could there be detected evidence of derma-

titis, conjunctivitis, or any other inflammatory process which might be attributed to the action of ultra-violet radiation. The other animals, Groups I, III, and V, remained in the dark room throughout the experiment. Group I served as a control for Groups III and V, and Group II, as the control for Groups IV and VI.

A summary of experimental procedures, according to groups, is given in the following table:

Group	Number of animals	Dark room	Ultra-violet irradiation started	Inoculated with *T. pallidum*	Termination of experiment
I	10	Nov. 27, 1928			June 15, 1929
II	10	Nov. 27, 1928	Dec. 5, 1928		June 15, 1929
III	10	Nov. 27, 1928		Dec. 18, 1928	June 15, 1929
IV	10	Nov. 27, 1928	Dec. 5, 1928	Dec. 18, 1928	June 15, 1929
V	10	Nov. 27, 1928		Jan. 24, 1929	June 15, 1929
VI	10	Nov. 27, 1928	Dec. 5, 1928	Jan. 24, 1929	June 15, 1929

All animals received some light from two other sources. For a brief period each day a 30 Watt amber tinted Mazda lamp was used in the dark room for cleaning cages, feeding, and making certain necessary observations. The animals were also exposed to diffuse filtered sunlight for a brief period once every 2 weeks when they were brought into the laboratory for bleeding purposes. During the remaining time all animals were kept in the dark room.

The mean values contained in Tables I to VI inclusive have been smoothed by the formula $\frac{a + 2b + c}{4}$ and are presented in the graphs (Text-figs. 1 to 3) in terms of per cent variation from a standard mean value, using for this purpose 2200 gm.

RESULTS

The results of the observations made in this experiment are presented in the form of tabulated summaries, Tables I to VIII, which are supplemented by a series of graphs, Text-figs. 1 to 3.

TABLE I

Gross Body Weight. Normal Dark Room

Date	Group mean	Smoothed net variation	Per cent variation
1928-29	*gm.*	*gm.*	
Nov. 30	1940	−260	−11.8
Dec. 6	2043	−167	− 7.6
Dec. 12	2088	−113	− 5.1
Dec. 20	2132	− 82	− 3.7
Dec. 28	2121	− 61	− 2.8
Jan. 5	2182	− 21	− 0.9
Jan. 12	2232	+ 17	+ 0.8
Jan. 18	2222	+ 32	+ 1.5
Jan. 25	2253	+ 59	+ 2.7
Feb. 5	2309	+105	+ 4.8
Feb 9	2350	+154	+ 7.0
Feb. 16	2409	+199	+ 9.0
Feb. 22	2431	+186	+ 8.8
Mar. 6	2273	+117	+ 5.3
Mar. 16	2290	+ 94	+ 4.3
Mar. 23	2325	+114	+ 5.2
Mar. 29	2315	+ 88	+ 4.4
Apr. 6	2197	+ 33	+ 1.5
Apr. 14	2225	+ 25	+ 1.1
Apr. 19	2262	+ 61	+ 2.8
Apr. 27	2295	+ 98	+ 4.5
May 11	2342	+138	+ 6.3
May 17	2355	+103	+ 4.7
May 25	2162	− 1	0
June 1	2120	− 58	− 2.6
June 8	2167	− 37	− 1.7
June 15	2200	0	0
Group mean.....	2231	+ 31	+ 1.4

Dec. 6 to Jan. 25—14.5.
Mean gain—13.2.
Final net gain—11.8.

TABLE II

Gross Body Weight. Normal Ultra-Violet

Date	Group mean	Smoothed net variation	Per cent variation
1928–29	*gm.*	*gm.*	
Nov. 30	1865	−335	−15.7
Dec. 6	1970	−194	− 8.8
Dec. 12	1998	−150	− 7.0
Dec. 20	2084	−133	− 6.0
Dec. 28	2103	− 77	− 3.5
Jan. 5	2216	− 10	− 0.4
Jan. 12	2228	+ 34	+ 1.5
Jan. 18	2265	+ 46	+ 2.1
Jan. 25	2236	+ 59	+ 2.6
Feb. 5	2322	+ 75	+ 3.4
Feb. 9	....	.....	.....
Feb. 16	2220	+104	+ 4.7
Feb. 22	....	.....	.....
Mar. 6	2453	+186	+ 8.8
Mar. 16	2420	+106	+ 4.8
Mar. 23	....	.....	.....
Mar. 29	....	.....	.....
Apr. 6	....	.....	.....
Apr. 14	2031	− 63	− 2.9
Apr. 19	....	.....	.....
Apr. 27	2065	−133	− 6.0
May 11	2109	− 81	− 3.6
May 17	2193	− 31	− 1.3
May 25	2180	− 47	− 2.1
June 1	2059	− 98	− 4.5
June 8	2112	−114	− 5.2
June 15	2063	−137	− 6.2
Group mean.....	2152	− 48	− 2.2

Dec. 6 to Jan. 25—18.3.
Mean gain—13.5.
Final net gain—9.5.

TABLE III

Gross Body Weight. Dark Room. T. pallidum Inoculated on December 12, 1928

Date	Group mean	Smoothed net variation	Per cent variation
1928–29	gm.	gm.	
Nov. 30	1902	−197	− 8.9
Dec. 6	1955	−119	− 5.4
Dec. 12	2180	− 98	− 4.5
Dec. 20	2000	− 63	− 2.9
Dec. 28	2230	+ 31	+ 1.3
Jan. 5	2240	+ 27	+ 1.2
Jan. 12	2342	+168	+ 7.6
Jan. 18	2387	+174	+ 7.9
Jan. 25	2357	+193	+ 8.8
Feb. 5	2397	+211	+ 9.6
Feb. 9	2422	+211	+ 9.6
Feb. 16	2405	+230	+10.5
Feb. 22	2415	+247	+11.2
Mar. 6	2485	+230	+10.5
Mar. 16	2405	+213	+ 9.7
Mar. 23	2425	+214	+ 9.7
Mar. 29	2417	+213	+ 9.7
Apr. 6	2400	+245	+11.2
Apr. 14	2425	+274	+12.4
Apr. 19	2470	+298	+13.5
Apr. 27	2500	+327	+14.9
May 11	2500	+321	+14.8
May 17	2567	+240	+10.9
May 25	2450	+108	+ 4.8
June 1	2295	+ 25	+ 1.1
June 8	2215	+ 6	+ 0.2
June 15	2200	0	0
Group mean.....	2333	+133	+ 6.0

TABLE IV

Gross Body Weight. Ultra-Violet. T. pallidum Inoculated on December 12, 1928

Date	Group mean	Smoothed net variation	Per cent variation
1928–29	*gm.*	*gm.*	
Nov. 30	1852	−248	−11.2
Dec. 6	1900	−183	− 8.7
Dec. 12	2017	−101	− 4.6
Dec. 20	2065	−132	− 6.0
Dec. 28	2127	− 87	− 4.3
Jan. 5	2135	− 56	− 2.4
Jan. 12	2180	− 21	− 0.9
Jan. 18	2212	+ 12	+ 0.5
Jan. 25	2245	+ 40	+ 1.8
Feb. 5	2260	+ 41	+ 1.8
Feb. 9	2212	+ 14	+ 0.6
Feb. 16	....	.....	.....
Feb. 22	2175	− 24	− 1.1
Mar. 6	....	.....	.....
Mar. 16	2134	− 66	− 3.0
Mar. 23	2093	− 87	− 3.9
Mar. 29	2142	− 67	− 3.0
Apr. 6	2156	− 53	− 2.4
Apr. 14	2134	− 71	− 3.2
Apr. 19	....	.....	.....
Apr. 27	....	.....	.....
May 11	2090	−108	− 4.9
May 17	....	.....	.....
May 25	....	.....	.....
June 1	2053	−147	− 6.7
June 8	....	.....	.....
June 15	....	.....	.....
Group mean.....	2115	− 85	− 3.9

TABLE V

Gross Body Weight. Dark Room. T. pallidum Inoculated on January 24, 1929

Date	Group mean	Smoothed net variation	Per cent variation
1928–29	*gm.*	*gm.*	
Nov. 30	1932	−268	−12.2
Dec. 6	2162	−178	− 8.1
Dec. 12	2222	+ 15	+ 0.7
Dec. 20	2255	+ 56	+ 2.4
Dec. 28	2295	+ 91	+ 4.1
Jan. 5	2325	+130	+ 5.9
Jan. 12	2380	+174	+ 7.9
Jan. 18	2412	+212	+ 9.6
Jan. 25	2447	+245	+11.1
Feb. 5	2477	+274	+12.4
Feb. 9	2497	+303	+13.8
Feb. 16	2542	+305	+13.9
Feb. 22	2442	+254	+11.5
Mar. 6	2387	+208	+ 9.5
Mar. 16	2417	+204	+ 9.4
Mar. 23	2395	+191	+ 8.7
Mar. 29	2357	+130	+ 5.9
Apr. 6	2312	+128	+ 5.8
Apr. 14	2332	+127	+ 5.8
Apr. 19	2332	+146	+ 6.6
Apr. 27	2391	+163	+ 7.5
May 11	2339	+148	+ 6.7
May 17	2322	+103	+ 4.7
May 25	2230	+ 50	+ 2.3
June 1	2218	+ 44	+ 2.0
June 8	2290	+ 90	+ 4.1
June 15	....	.....	.....
Group mean.....	2335	+135	+ 6.1

TABLE VI

Gross Body Weight. Ultra-Violet. T. pallidum Inoculated on January 24, 1929

Date	Group mean	Smoothed net variation	Per cent variation
1928-29	*gm.*	*gm.*	
Nov. 30	1922	−258	−11.5
Dec. 6	2061	−168	− 7.6
Dec. 12	2086	− 96	− 4.2
Dec. 20	2183	− 39	− 1.7
Dec. 28	....	.....	.....
Jan. 5	2194	− 2	− 0.1
Jan. 12	2222	+ 22	+ 1.0
Jan. 18	2250	+ 48	+ 2.2
Jan. 25	2269	+ 75	+ 3.4
Feb. 5	2303	+ 81	+ 3.7
Feb. 9	2250	+ 68	+ 3.1
Feb. 16	2272	+ 69	+ 3.1
Feb. 22	2266	+ 65	+ 3.0
Mar. 6	2267	+ 69	+ 3.1
Mar. 16	2277	+ 74	+ 3.4
Mar. 23	....	.....	.....
Mar. 29	2275	+ 45	+ 2.0
Apr. 6	....	.....	.....
Apr. 14	....	.....	.....
Apr. 19	2155	+ 40	+ 1.9
Apr. 27	....	.....	.....
May 11	....	.....	.....
May 17	2025	− 48	− 2.2
May 25	....	.....	.....
June 1	2002	−148	− 6.7
June 8	1922	−212	− 9.6
June 15	....	.....	.....
Group mean.....	2168	− 32	− 1.5

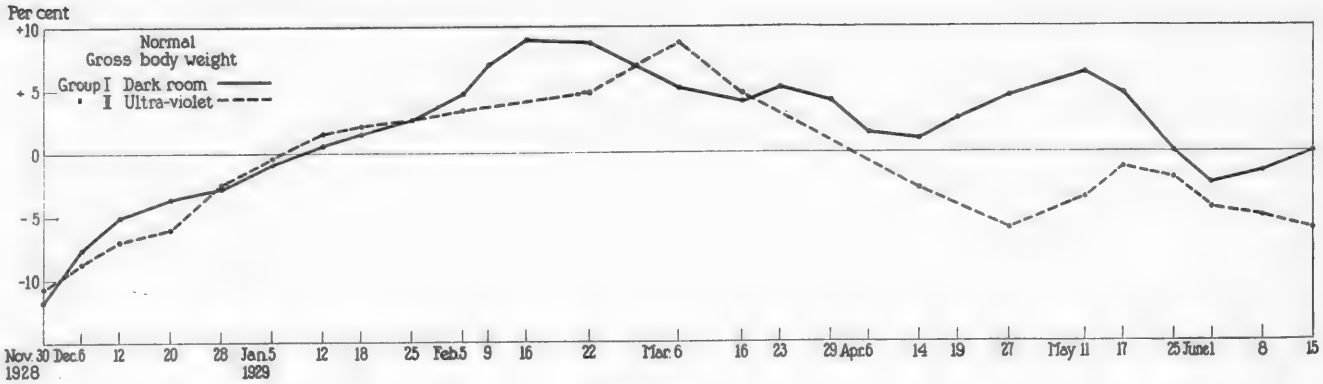
TABLE VII

Per Cent Gain from Initial Weight

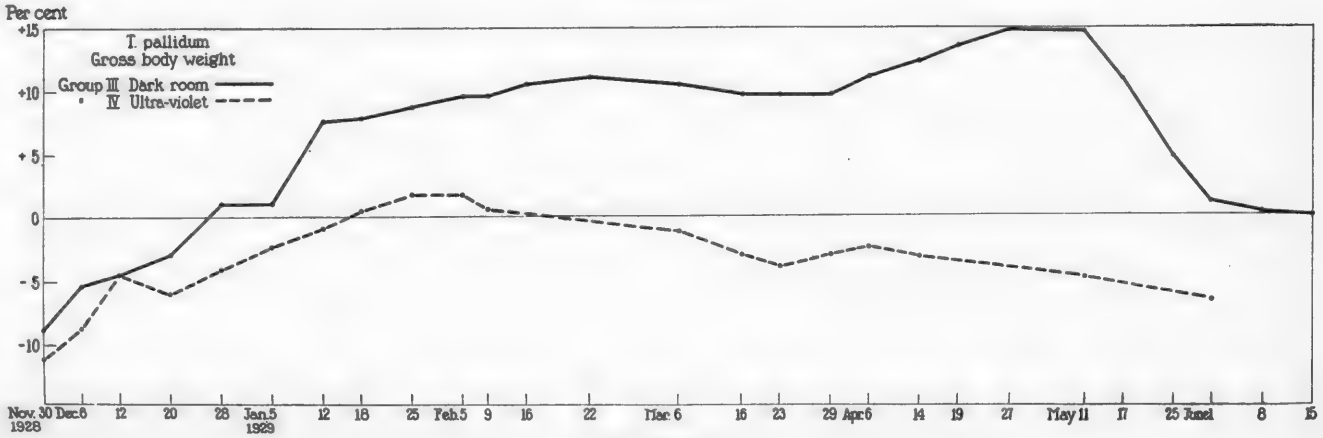
Dark room				Ultra-violet			
Group	Jan. 25, 1929	Mean	Final	Group	Jan. 25, 1929	Mean	Final
I	16.1	15.0	13.4	II	19.9	15.4	10.6
III	24.8	22.6	15.1	IV	21.2	14.2	10.8
V	26.6	20.8	18.5	VI	18.1	12.7	0.0

TABLE VIII

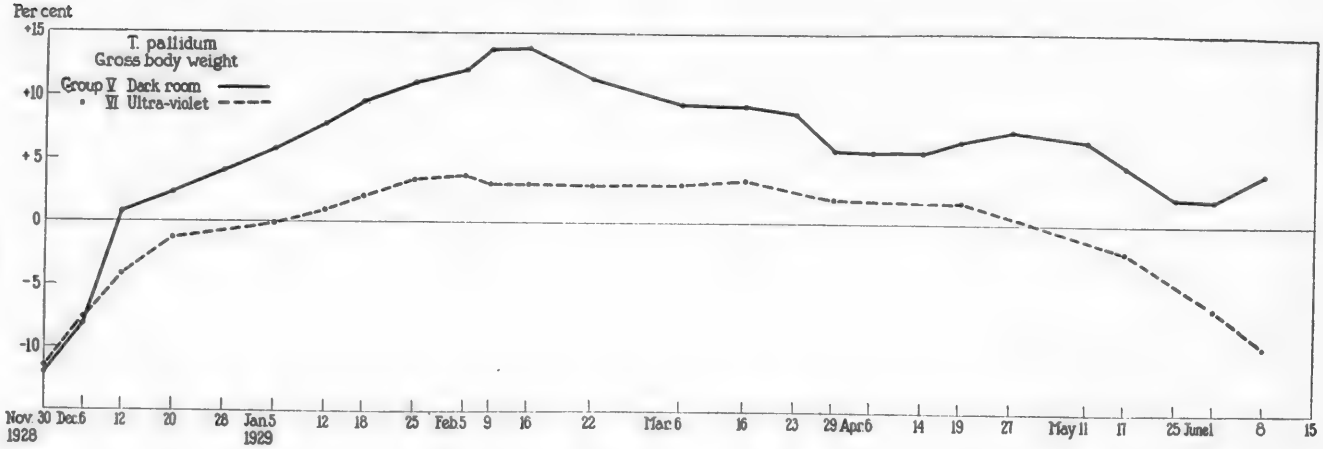
Dark room		Ultra-violet	
Group	Survivals	Group	Survivals
I	10	II	6
III	10	IV	8
V	9	VI	9



TEXT-FIG. 1



TEXT-FIG. 2


Per cent
+15
+10
+5
0
-5
-10
T. pallidum
Gross body weight
Group V Dark room
VI Ultra-violet
Nov. 30 1928
Dec. 6
12
20
28
Jan. 5 1929
12
18
25
Feb. 5
9
16
22
Mar. 6
16
23
29
Apr. 6
14
19
27
May 11
17
25
June 1
8
15

TEXT-FIG. 3

DISCUSSION AND CONCLUSIONS

In taking up the discussion of the results recorded above, attention should be directed to the fact that in these experiments the only light of any consequence which was received by the irradiated animals was that derived from the quartz mercury lamp. These experiments differ, therefore, from others in which animals receiving unfiltered or diffuse filtered sunlight are given an additional ultra-violet irradiation. A further condition to which attention should be called is the fact that during the course of these experiments, there was a marked deterioration of the lamp used, so that the energy delivered during the latter part of the experiment was much less than at the beginning. It is regretted that the actual change and the rate of decrease can not be given accurately. But throughout the entire period of the experiment, all animals lived in a room from which all extraneous light was excluded, the only evironmental variation being the exposure of Groups II, IV, and VI to ultra-violet radiation for an hour each day at a distance of 4.5 meters.

Considering the results for normal animals first, it will be seen by a comparison of the figures for body weight that from the beginning of the experiment on November 30, 1928, until January 25, 1929, both Groups I and II showed a gradual increase in body weight. Following this, the dark group exhibited a rather sharp increase in weight reaching a maximum on February 16. The increase in the ultra-violet group of animals was more gradual, their maximum weight being attained on March 6. From these high values both groups showed a loss in weight, the group exposed to ultra-violet radiation losing more than animals living in total darkness. After March 16 and throughout the remainder of the experiment, the general level of body weight of the ultra-violet group was below that of the dark group. The latter group exhibited a secondary increase in weight during the first week of May, this same increase being reflected in the ultra-violet animals although at a much lower level. From these levels, both groups decreased in weight, the ultra-violet animals showing a more gradual decline while the dark group showed a slight increase in weight during the last 2 weeks of the experiment.

The difference between the two groups of animals with respect to

susceptibility to intercurrent disease (Table VIII) is in agreement with the results obtained for body weight. This is clearly shown by the occurrence of 4 deaths among the irradiated animals and none among those which received no ultra-violet light. Postmortem examination showed that death was due in all cases to pneumonia.

The effects of irradiation on syphilitic rabbits were essentially the same as on normal animals. The animals of Groups III and IV (Tables III and IV; Text-fig. 2) were inoculated with the Nichols strain of *T. pallidum* on December 24, 1928. All other conditions were the same as for Groups I and II. Except for the values obtained on December 12, at which time both groups coincided, the gross body weights for animals exposed to ultra-violet radiation was far below that maintained by animals living in total darkness. On May 6, there was a difference of 20 per cent between the mean values obtained for these two groups of animals. While the course of syphilis in the two groups of animals was moderately severe, yet no deaths were recorded as resulting from the specific infection. However, at the end of the experiment, all animals of Group III were alive while 2 died in Group IV, deaths being due to pneumonia.

The animals in Groups V and VI were inoculated on January 24, 1929, with the same strain of *T. pallidum* as were Groups III and IV. In general, it can be stated that the syphilitic infection in these animals was less severe than that found in Groups III and IV. The mortality rate from non-specific infection was the same in both Groups V and VI.

From the beginning of the experiment until December 6, the animals in Group VI showed a higher gross body weight than those in Group V, but from this time on the animals in Group VI maintained a level below that of Group V. In these two groups of animals, it will be noted that the difference in weight is not as great as that found to exist between Groups III and IV.

If the gain in weight is calculated in per cent of the original or first recorded weights (Table VII), essentially the same results are demonstrable. At the end of the first 7 weeks of the experiment, January 25, 1929, the animals in Group II showed a greater per cent gain in weight than did the control animals in Group I. It will also be noted that the mean per cent gain was slightly higher for animals exposed to ultra-violet radiation than for those animals in the dark room. The final

weight for the ultra-violet group was less than for those animals in the dark room. In the 4 other groups the per cent gain was less in animals exposed to ultra-violet light than in those remaining in total darkness; in Group VI the final weight was exactly the same as the original.

Another factor which influences all calculations is the mortality rate among these 6 groups of animals (Table VIII). Seven deaths occurred among animals remaining in the dark room. The deaths in each group were due to pneumonic infections.

SUMMARY AND CONCLUSION

In summarizing the results obtained for these 6 groups of animals, the following conclusions may be drawn:

Normal rabbits living in total darkness and exposed to ultra-violet radiation at regular intervals showed a more rapid rate of increase in weight than animals living under the same condition, but after an initial period of rapid increase, the irradiated animals maintained a lower body weight than those living in the dark.

Under the same conditions, animals inoculated with *T. pallidum* and exposed to ultra-violet light maintained a lower weight than the corresponding control groups living in total darkness.

Furthermore, the mortality rate from pneumonic infection was found to be greater in animals exposed to ultra-violet radiation than in those living entirely in the dark.

It is evident, therefore, that, under the conditions given, ultra-violet radiation was detrimental rather than beneficial.

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A STUDY OF THE RESISTANCE OF NORMAL HUMAN BEINGS TO RECENTLY ISOLATED STRAINS OF PATHOGENIC PNEUMOCOCCI*

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(Received for publication, May 14, 1930)

That man must be fairly resistant to the pneumococcus is attested by the relative infrequence of known contact infection among those attending pneumonia patients, despite the fact that under such conditions pneumococci are probably often inhaled in considerable numbers. When contact infection has been observed it is almost always with Pneumococcus Types I and II from which it might be inferred that these two types are the most virulent for man. But we have no definite information as to the relative human virulence of different strains or types of pathogenic pneumococci and but little data concerning variations in individual susceptibility to a single strain.

While a direct approach to this problem is not possible for obvious reasons, an indirect method of study has become available as a result of experimental observation in animals. It was found that the resistance of an animal to pneumococcus infection was paralleled very closely by the pneumococcidal power of the blood. When the serum and leucocytes of a single species, the rabbit, were tested against pneumococci of varying rabbit virulence, the pneumococci of low virulence were destroyed in considerable numbers while those of high virulence grew readily in the serum-leucocyte mixtures. Again the serum and leucocytes of young rabbits which are highly susceptible to pneumococci of low virulence for the adult rabbit, were found to possess no pneumococcidal action whatsoever. There was found to be a general quantitative relationship between the killing dose of pneumococci and the pneumococcidal power of the animal's blood (1, 2).

* Reported in brief in the *Proceedings of the Institute of Medicine of Chicago*, 1929, **7**, 224.

Method and Materials

The pneumococcidal tests were carried out as already described (1,3) employing quantitated mixtures of serum, leucocytes, and pneumococci in sealed tubes which were agitated during incubation. Pooled normal serum in each test consisted of a mixture of serum secured from four to seven persons.[1] Human leucocytes were obtained from Group I (Jansky) donors. Serum and leucocytes from the same person were often used in testing single individuals. The strains of pneumococci employed were those freshly isolated from cases of lobar pneumonia. Frequently they were used after only one transfer in broth. The standard suspension was one billion organisms per cubic centimeter. Tests for opsonins and aglutinins were made as detailed in a previous study (4).

We are much indebted to Miss Georgia Cooper for diagnostic sera which enabled us to classify strains of atypical Type II and Group IV pneumococci.

EXPERIMENTAL

Pneumococcidal Action of Normal Human Serum-Leucocyte Mixtures

The tests were carried out as shown in Table I. From one to four pneumococcus strains were tested at a time and the tests were repeated in order to secure comparative results. It is seen in this experiment that the pneumococci of all four strains were killed in relatively large numbers by the normal human serum and leucocytes. Observations on 26 strains studied in this way are summarized in Table II. While considerable variation in susceptibility to the killing action of the serum-leucocyte mixtures was found to exist among the different members of the series, none were able to survive or grow in quantities less than 10^{-5} of the standard suspension. The variations in growth capacity appeared to be independent of type nor did it seem to signify whether the pneumococci were isolated from the blood, lung or sputum, in recovering or fatal cases, early or late in the disease. An interpretation of these results on the basis of the much more complete observations in animals cited above, indicates that normal human beings as a group possess a considerable degree of natural immunity against pathogenic pneumococci.

[1] It was not possible to get an accurate past history from all of them as to antecedent occurrence of pneumonia but judging by a number of tests on persons of known negative past history, the inclusion of individuals who may have had pneumonia years previously could have made no material difference in the results.

TABLE I

Pneumococcidal Action of Normal Human Serum and Leucocytes for Recently Isolated Pneumococci

Human serum* 0.2 cc. + leucocyte suspension 0.1 cc. + pneumococcus suspension 0.1 cc. + gelatin Locke's solution 0.1 cc.

Type	Approximate number of pneumococci	Growth as shown by methemoglobin formation at			Survival of pneumococci at 72 hours
		15 hrs.	42 hrs.	72 hrs.	
	1,000,000(10^{-3})	+	+++	++++	+
(Sputum)	100,000(10^{-4})	0	0	0	0
	10,000(10^{-5})	0	0	0	0
	1,000(10^{-6})	0	0	0	0
	100(10^{-7})	0	0	0	0
II	1,000,000(10^{-3})	0	+	+++	+
(Blood)	100,000(10^{-4})	0	+	+++	+
	10,000(10^{-5})	0	0	0	0
	1,000(10^{-6})	0	0	0	0
IIa	1,000,000(10^{-3})	0	++	++++	+
(Blood)	100,000(10^{-4})	0	0	0	0
	10,000(10^{-5})	0	0	0	0
	1,000(10^{-6})	0	0	0	0
III	1,000,000(10^{-3})	0	+	+++	+
(Lung)	100,000(10^{-4})	0	0	+++	+
	10,000(10^{-5})	0	0	0	0
	1,000(10^{-6})	0	0	0	0
Controls with serum only**					
I	100(10^{-7})	++++			+
II	100(10^{-7})	++++			+
IIa	100(10^{-7})	+++			+
III	100(10^{-7})	+			+
Plates of 10^{-7} dilution of the pneumococcus suspensions					
I	No. of colonies			114	
II	" " "			50	
IIa	" " "			41	
III	" " "			90	

* Pooled serum.

** 0.1 cc. Laked red blood cells added.

The virulence of the pneumococci for mice was found to parallel in a general way their capacity to grow in the human serum-leucocyte

TABLE II

Pneumococcidal and Opsonic Action of Normal Human Serum and Leucocytes for Recently Isolated Pneumococci

Case	Type	Pneumococci isolated		Outcome of disease	Action of normal serum and leucocytes		
		From	Day of disease		Pneumococcidal Largest amt. susp. killed	Opsonic	Agglutination
R. S.	I	Sputum	10th	Rec'd	10^{-4}	−	+
L. R. E.	I	Blood	4th	Died	10^{-4}	−	−
J. L.	I	Sputum	4th	Rec'd	10^{-4}	+	++
A. I.	I	Sputum	6th	Rec'd	10^{-3}	−	−
W. Mc.	I	Lung	5th	Rec'd	10^{-3}	−	−
J. T. A.	I	Sputum	1st	Rec'd	10^{-5}	−	−
J. V.	II	Blood	2nd	Died	10^{-5}	++++	++++
S. P.	II	Blood	7th	Died	10^{-5}	−	++++
C. McN.	II	Sputum	3rd	Died	10^{-5}	+	++
M. M.	II	Sputum	7th	Rec'd	10^{-4}	+	++
N. S.	II	Sputum	4th	Rec'd	10^{-3}	++++	++++
J. C.	IIa	Sputum	11th	Rec'd	10^{-4}	−	++
R. E.	IIa*	Lung	1st	Rec'd	10^{-5}	++	−
M. L. B.	IIa*	Lung	4th	Rec'd	10^{-4}	+++	++++
R. McN.	IIa	Sputum	7th	Died	10^{-4}	−	++++
Y. B.	IIa	Blood	3rd	Rec'd	10^{-4}	++++	+++
H. F. L.	IIa*	Sputum	5th	Rec'd	10^{-4}	−	−
F.	IIa	Sputum	2nd	Rec'd	10^{-4}	+	+
H. C.	III	Lung	1st	Rec'd	10^{-5}	+++	++
V. C.	III	Blood	3rd	Died	10^{-4}	−	−
A. Z.	III	Lung	3rd	Rec'd	10^{-3}	−	−
K. H.	III	Sputum	2nd	Rec'd	10^{-5}	−	−
J. B.	IV	Sputum	5th	Rec'd	10^{-5}	+++	++
S. B.	IV	Sputum	4th	Rec'd	10^{-3}	++++	++++
F. V.	IV	Lung	6th	Rec'd	10^{-3}	−	−
H. F. P.	IV	Blood	3rd	Died	10^{-4}	−	−
E. J.	IV	Sputum	1st	Rec'd	10^{-5}	−	−

−, not done.

* Type V Cooper.

mixtures. Those able to grow in the serum and leucocytes in quantities as small as 10^{-5} were highly lethal for mice, killing in amounts of

10^{-7} of the standard suspension; while the strains which were killed in amounts of 10^{-3} of the standard suspension were of decidedly lower mouse virulence.[2]

Tests for the opsonic and agglutinative action of normal pooled human serum against these strains showed much more marked individual variation than did the pneumococcidal. While all those tested were agglutinated and phagocyted under the influence of the normal serum, the degree to which these actions occurred differed considerably with the several strains. These fluctuations might be accounted for, at least to a certain degree, by the fact that the technique for opsonic and agglutinative effect is much less susceptible to quantitative measure than is the pneumococcidal test.

Individual Variations

When the serum and leucocytes from a single individual were tested with several types of pneumococci, striking variations in pneumococcidal action against different organisms were often observed (Table III). This ranged from marked effect on one organism to none against another. Table IV gives a summary of the tests in 15 normal individuals using three to four types in each case.[3] Two experiments included three different individuals each, the first six in the table, tested against all four types at the same time. Tests on individuals T. B., R. G., and G. S. were also carried out in one experiment. The plus signs indicate degree of pneumococcidal effect. $+$ = killing of 10^{-7} of the standard suspension; $++$ = 10^{-6}, etc. The letters following the signs designate the strain of organism employed. It will be noted (Table IV) that in certain instances, the serum and leucocytes of one individual show marked killing power for an organism against which the blood elements of another person are without any effect.

[2] This relationship did not seem to hold as closely when the tests were repeated months or a year following isolation. Certain strains, notably those of Type II, had lost much of their growth capacity in human serum-leucocyte mixtures but still retained their mouse virulence.

[3] In the majority of these experiments, the sera from different persons were used with leucocytes from a single individual. This was possible because of the lack of any detectable individual variations in the activity of the leucocytes of normal human beings in the above tests.

These differences are of such a magnitude as to preclude their being due to errors in technique. Furthermore they were corroborated by

TABLE III

Variations in Pneumococcidal Activity of a Normal Human Being's Serum and Leucocytes for Several Types of Pneumococci

Serum 0.2 cc. + leucocyte suspension 0.1 cc. + pneumococcus suspension 0.1 cc. + gelatin Locke's solution 0.1 cc.

Type	Amount of pneumococcus suspension	Growth as shown by methemoglobin formation at			Survival of pneumococci at 72 hours
		15 hrs.	46 hrs.	72 hrs.	
I	10^{-3}	+	+++	++++	+
	10^{-4}	0	0	0	0
	10^{-5}	0	0	0	0
	10^{-6}	0	0	0	0
	10^{-7}	0	0	0	0
IIa (V Cooper)	10^{-4}	++++			+
	10^{-5}	++++			+
	10^{-6}	++++			+
	10^{-7}	++++			+
II	10^{-4}	0	0	0	0
	10^{-5}	0	0	0	0
	10^{-6}	0	0	0	0
	10^{-7}	0	0	0	0
Controls with serum only					
I	10^{-7}	++++			+
IIa	10^{-7}	+	++++		+
II	10^{-7}	++++			+
Plates of 10^{-7} dilution of the pneumococcus suspensions					
I	No. of colonies				114
IIa	" " "				46
II	" " "				14

repeated tests which gave essentially the same results. All persons tested possessed pneumococcus-killing blood properties of some degree for at least one type of organism while the majority showed them for

two or more.[4] The only type for which every individual exhibited pneumococcidal action was Type II.

It was now of interest to determine whether these varying individual responses to different pneumococci depended on the particular strain used or on the type. Accordingly the serum and leucocytes of five of

TABLE IV

Variations in Pneumococcidal Activity of Serum and Leucocytes of Normal Individual Human Beings for Several Types of Pneumococci

Normal individual	Pneumococcidal activity of blood for types							
	I		II		III		IIa (V Cooper)	
M. A. C.	0	(El.)	++++	(Vo.)	+++++	(Ca.)	0	(En.)
W. R.	0	(El.)	++++	(Vo.)	++++	(Ca.)	0	(En.)
D.	0	(El.)	+++++	(Vo.)	+++	(Ca.)	++	(En.)
O. H. R.	+++	(El.)	++++	(Pa.)	0	(McT.)	+	(En.)
E. S. L.	++++	(El.)	++++	(Pa.)	++	(McT.)	+++++	(En.)
H. V. S.	+	(El.)	++	(Pa.)	0	(McT.)	0	(En.)
E. E. T.	0	(El.)	+++++	(Pa.)	+++	(McT.)	−	
N.	++++	(El.)	−		−		+++++	(En.)
T. H.	++++	(St.)	++++	(Me.)	−		0	(En.)
T. B.	+++	(A_8)	+++	(Vo.)	++++	(Cl.)	−	
R. G.	++	(A_8)	++++	(Vo.)	++++	(Cl.)	−	
							Group IV	
G. S.	0	(A_8)	++++	(Vo.)	0	(Cl.)	0	(Ho.)
F. R.	+++	(A_8)	++++	(Vo.)	++	(Cl.)	−	
T. E. F.	+++++	(A_8)	+++	(Vo.)	+++++	(Zl.)	−	
J. G.	+++++	(A_8)	+++	(Vo.)	+++++	(Zl.)	−	

+, killing of 10^{-7} of the standard suspension. ++, killing of 10^{-6} of the standard suspension. +++, killing of 10^{-5} of the standard suspension. Etc.

Letters in parentheses indicate strain of pneumococcus.

−, not done.

the above persons were tested with three separate strains of each type. Two of the individuals, E. S. L. and T. B., showed a well marked killing

[4] In an earlier paper on natural immunity to pneumococci (4) human beings were included in the group of mammals relatively susceptible to pneumococcus infection. This erroneous conclusion resulted from the finding that the serum of the several human beings tested showed no opsonic action for the single strain of pneumococcus used, a virulent Type I.

action for all of the types used. The results recorded in Table V seem to indicate that type is the conditioning factor. While there occurred variations in degree of reaction between the different strains yet by and large these were less than the differences between types. However, certain strains showed more growth activity in the serum-leucocyte mixture than others. Two of these, R., a Type I and McT., a

TABLE V

Comparative Pneumococcidal Power of Individuals' Serum and Leucocytes for Different Strains of Several Pneumococcus Types

Normal individual	Pneumococcus type	Pneumococcidal activity for different strains		
M. A. C.	I	0 (El.)	0 (Ri.)	+ (A_8)
	II	++++ (Vo.)	++++ (Pa.)	+++ (Me.)
	III	++++ (Ca.)	+++ (McT.)	++++ (B_1)
	IIa*	0 (En.)	+ (Br.)	+ (LV.)
W. R.	I	++ (El.)	0 (Ri.)	++ (A_8)
	II	+++++ (Vo.)	+++++ (Pa.)	+++ (Me.)
	III	++++ (Ca.)	++ (McT.)	++++ (B_1)
	IIa*	0 (En.)	++ (Br.)	+ (LV.)
E. S. L.	I	++++ (El.)	+ (Ri.)	+++ (B_{10})
	II	++++ (Vo.)	+++++ (Pa.)	++++ (Me.)
	III	+++++ (Ca.)	+ (McT.)	+++++ (B_1)
	IIa*	+++ (En.)	++++ (Br.)	+++++ (LV.)
T. B.	I	+++++ (McC.)	++++ (Al.)	++ (A_8)
	II	+++ (Vo.)	+++++ (Pa.)	+++++ (Me.)
	III	++++ (Cl.)	+++++ (Zl.)	++++ (Ha.)
G. S.	I	0 (McC.)	+ (Al.)	+ (A_8)
	II	+++ (Vo.)	++ (Pa.)	++ (Me.)
	III	+ (Cl.)	0 (Zl.)	0 (Ha.)

* Type V Cooper.

Type III were consistently inhibited to a decidedly less degree than were the other members of the same type, which suggests that these strains were of higher human virulence.[5]

[5] They were not included with the freshly isolated strains as they had been secured a number of months earlier.

DISCUSSION

The above study while comprising only a relatively small number of pneumococcus strains, affords no evidence than any one type of pneumococcus is of higher virulence for human beings than the others. In fact, the pneumococcidal potency of the pooled normal human serum and leucocytes exhibits a surprising uniformity for the several types of pneumococci. Variations shown for different strains were not confined to any one type. Possibly the inclusion of a larger number of the members of Group IV would show that these organisms have, as a class, a lesser growth capacity in normal human serum and leucocytes than the so-called fixed types, although the recent reclassification of Group IV and Type II atypical pneumococci by Cooper, Edwards and Rosenstein (5) indicates that a considerable percentage of these organisms are to be considered as highly pathogenic as the members of Types I, II, III.

Clough's (6) findings that the serum of certain normal human beings showed mouse protective properties which differed in degree against the several types of pneumococci suggested that natural humoral antipneumococcus immunity, in man, is characterized by fairly wide individual variations. Our findings support this supposition and further indicate the nature of man's natural humoral immunity.

It is, of course, recognized that resistance to pneumococcus infection, particularly that of the lungs, is dependent upon factors both local and general in addition to the pneumococcus-killing power of the blood. But with the present lack of appropriate methods we have no means of estimating the functional activity of these other defense processes such as the eliminatory function of the lungs, the phagocytic activity of the fixed macrophages, etc.; of knowing which is the principal and which the secondary means of defense. However, in view of the studies on experimentally infected animals referred to earlier, in which a close correspondence was found to exist between resistance to pneumococcus infection and the pneumococcidal power of their blood it seems fair to assume that the presence of this same property in the blood of human beings indicates their capacity to combat invasion by pneumococci in any part of the body to which the blood has free access.

In relation to the high mortality of lobar pneumonia caused by

Pneumococcus Type II it was surprising to find that the group of 15 normal individuals tested showed a more constant pneumococcidal power for organisms of this type than for those of other types. That this result is not to be attributed to the employment of strains of relatively low human pathogenicity is indicated by the fact that two of them were isolated from the blood of fatally terminating cases, one a fulminating case dying within 48 hours. However, there is some evidence to suggest that the virulence of these Type II strains for human beings may have diminished before being employed in the tests with normal individuals. When first isolated the two blood strains were killed by the pooled serum-leucocyte mixtures in quantities of the suspension only to 10^{-5}. Two months later they were killed by the blood of normal individual human beings in quantities of 10^{-4} or 10^{-3} yet their mouse virulence appeared to be unaltered.

That an individual should exhibit such marked variations in pneumococcidal activity for the different types of pneumococci is of particular interest in view of our previous findings in other pneumococcus resistant mammals. While an occasional deviation was observed in tests on dogs and cats it was slight in degree compared to those found in this study. The reactions of the human serum-leucocyte mixtures are much more comparable in this respect to those of a pneumococcus-susceptible animal such as the rabbit which shows marked individual fluctuations both in resistance to pneumococcus infection and in concentration of natural humoral immune bodies for different pneumococcus types. Whether or not these apparently specific reactions of the human blood elements depend on the presence of distinct opsonins for the several types of pneumococci we have not determined. Our knowledge of the nature of this reaction suggests that such is the case (4). Sia's recent finding of specific opsonins and mouse protective substances in normal pig serum against Pneumococcus Types I, II and III lends support to this view (7, 8).

There has appeared as this manuscript was about to be sent for publication a paper by Ward (9) dealing with the pneumococcidal power of the defibrinated blood of normal persons for different types of pneumococci. While Ward's technique varied considerably from ours and no mention is made of the source or time of isolation of the pneumococcus cultures used, his findings of a wide variability in blood

pneumococcidal activity between several individuals for a given type and in a single person for the three types of pneumococci agree essentially with our observations on these points.

SUMMARY

With a view to obtaining information as to the virulence of pneumococci for human beings a study was made of the pneumococcidal action of normal human serum-leucocyte mixtures for freshly isolated strains of pathogenic pneumococci. It was found that human beings as a group showed well marked pneumococcus destroying power in their blood for all types of organisms studied. Individuals, however, exhibited wide variations in their reactions against the different types. These ranged from marked killing effect for one type of pneumococcus to none or slight against another. While reactions against different strains within the type often varied considerably this difference was less, on the whole, than that between types. An interpretation of these findings in the light of previous animal experiments in which actual determination of resistance to pneumococcus infection was made leads to the inference that human beings in general possess a considerable degree of natural immunity to all types of pneumococci but that individuals may be relatively susceptible to one or more types and at the same time resistant to others; also that pathogenic strains of pneumococci vary much in their virulence for man.

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THE ORIGIN AND FATE OF TWO TYPES OF MULTINUCLEATED GIANT CELLS IN THE CIRCULATING BLOOD

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PLATES 10 AND 11

(Received for publication, March 13, 1930)

In the course of studies in the experimental production of giant cells in the tissues, reported by Doan, Sabin and Forkner (1), the writer discovered a procedure by which giant cells could be made to appear in the blood stream. This new material gave valuable evidence concerning the formation of giant cells, more especially of those of the so called foreign body and epithelioid types.

As far as can be ascertained, no one has hitherto reported a method to induce an appearance of giant cells in the blood, and no such cells have been found there, except megakaryocytes, which were reported by Oelhafen (2) and Naegeli (3) in 1914, by Minot (4) in 1922, by Sabin (5) in 1923, and by others. The usual condition in which they have been found is myelogenous leucemia.

Haythorn (6), in a recent review, has classified giant cells as follows: (a) Langhans' giant cells, (b) foreign body giant cells, (c) osteoclasts, (d) megakaryocytes, (e) muscle giant cells, (f) giant cells of nervous tissue, and (g) true tumor giant cells. It is relative to the first two of these types that this paper is concerned.

As early as 1868 Langhans (7), working with fresh tissues, demonstrated large multinucleated cells obtained from tuberculous lesions. For the most part the nuclei were arranged around the periphery of the cell, were usually round or oval, had sharp outlines and generally contained nucleoli. He stated that the protoplasm of such a giant cell was pale, homogeneous, or finely granular, with the center usually clear. This type of cell has since been designated the "Langhans' giant cell." However, some confusion has arisen because he also described another type of giant cell which had nuclei distributed throughout the cytoplasm.

For reasons given by Doan, Sabin and Forkner (1), the term "*epithelioid giant cell*" has been proposed to replace the often misunderstood term of Langhans' giant cell. This concept of the "epithelioid giant cell" is based on new information obtained through the methods of studying living cells with supravital (Simpson, 8)

(Sabin, 5) and vital stains. It was first demonstrated by Sabin, Doan and Cunningham (9) that the epithelioid cell is derived from the monocyte. The supravital technique showed that in the monocyte, surrounding its centrosphere, is a characteristic rosette of vacuoles, stainable in the living state with neutral red. An accentuation of this normal structure of the monocyte produces an epithelioid cell. Cunningham, Sabin, Sugiyama, and Kindwall (10) then confirmed the findings of other investigators that the Langhans' giant cell is a multinucleated epithelioid cell.

In general there have been two opinions concerning the types of cells under discussion. One group of investigators, among them Ziegler (11), Kockel (12), Hektoen (13), Maximow (14), and Medlar (15), believe that the Langhans' giant cell is merely a foreign body giant cell occurring in tuberculosis. Krückmann (16) studied giant cells in tuberculosis, about parasites and foreign materials, and in tumors. He concluded that microscopic means were not available by which foreign body giant cells and Langhans' giant cells could be distinguished. Langhans (7), Jacobson (17), Lubarsch (18) and others, however, believe that the giant cell of tuberculous tissue can be distinguished from all other kinds.

Concerning the mode of origin of the foreign body and epithelioid giant cells there has been some disagreement. Weigert (19), Baumgarten (20), Bakács (21), Lubarsch (18), and others were of the opinion that giant cells of tuberculous lesions were formed by continued nuclear division. Krauss (22), Mallory (23) and Wells (24) are among those who favor fusion of individual cells as the explanation for the formation of the types of cells under discussion. Lewis and Webster (25) in 1921 believed that epithelioid giant cells were formed by amitosis of the nucleus, but a later paper by Lewis (26) takes the opposite view, that they are formed by fusion of epithelioid cells.

Materials and Methods

The experiments reported in this communication have been carried out with the use of the methods of supravital and vital staining. Because of some recent minor modifications in the methods used in Dr. Sabin's laboratory, the solutions of dyes employed and their method of preparation will be described in detail.

We are now using six different solutions for our routine work. Solution 1 is prepared by dissolving 125 mg. of vital neutral red (Grübler) in 50 cc. of neutral absolute ethyl alcohol. This makes a 0.25 per cent solution which is saturated. It may be stated here that neutral red (Ehrlich) certified for use in vital staining as prepared by the National Aniline and Chemical Company, New York, is a thoroughly satisfactory dye which can be substituted for neutral red (Grübler). Solution 2 is prepared by dissolving 125 mg. of vital Janus green (National Aniline or Grübler) in 62.5 cc. of neutral absolute ethyl alcohol, thus making a 0.20 per

cent solution which likewise is saturated. By adding 50 drops of Solution 1 to 10 cc. of neutral absolute ethyl alcohol, we prepare Solution 3. Solution 4, a mixture of neutral red and Janus green, is made by adding 2 drops of Solution 2 to 3 cc. of Solution 3.

For the study of blood or other tissues, where cells to be stained are relatively few in number, we prepare slides in the manner described by Sabin (5). This is accomplished as follows: (a) by means of a pipette, flood the upper surface of a slide chemically clean with Solution 4; (b) drain excess of dye back into bottle by holding slide in an upright position; (c) absorb the excess of dye on the dependent edge of the slide with blotting material; (d) evaporate the remaining alcohol on the slide by immediately holding it over a piece of wire gauze under which a gas flame is burning. There is no objection to burning the excess of alcohol from the slide. The stained surface of the slide is then marked with a wax pencil and the slides are stored for use. Some investigators in the past have experienced difficulties in obtaining an even distribution of the dye over the slide, particularly when there is a high degree of humidity in the atmosphere. Preparing the slides as described, in artificially dried air, obviates these troubles, leaving a thin, even film of the dye.

For study of bone marrow, lymph nodes, or other tissues where there is an abundance of colorless cells to be stained, two more solutions are required. Solution 5 is prepared by adding 150 drops of Solution 1 to 10 cc. of neutral absolute ethyl alcohol. Solution 6, a mixture of neutral red and Janus green, is made by adding 8 drops of Solution 2 to 3 cc. of Solution 5. Thus Solution 6 contains approximately three times the amount of the dyes as Solution 4. Slides are prepared with Solution 6 in precisely the same manner as with Solution 4. The latter solutions, 4 and 6, being mixtures of neutral red and Janus green, deteriorate after about 24 to 48 hours and must be freshly prepared each time they are needed. The remaining solutions are stable and are best kept in glass stoppered bottles in a cool, dark place. Care must be exercised to make sure that the pipettes and bottles used are free of alkali. If an excess of alkali is present, the neutral red solution will become a muddy yellow color.

Blood films in these supravital studies are prepared by obtaining a drop of blood on a clean coverslip and letting it fall gently on the slide prepared from Solution 4. The film is quickly rimmed with vaseline (salvoline) and in the course of from 3 to 5 minutes is ready for study. The films are best studied in a constant temperature box at 38° or 39°C. For the study of the cells of organs such as the omentum or subcutaneous tissues, thin films of the intact tissue may be spread over slides prepared with Solution 6. If the organs are too dense or too thick to be spread intact over a slide, the cut surface of the fresh tissue, for example, lung, lymph nodes, etc., is scraped with a sharp scapel and the tissue accumulating on the knife blade is placed on a slide prepared from Solution 6. If the tissue is very dry a drop of plain normal salt solution or normal salt solution moderately colored with neutral red may be added to suspend the cells. A coverslip is then applied as above and is rimmed with vaseline. Material prepared in this way provides a thin enough film so that the individual living cells can be studied. For our

purposes, tissues fixed in Helley's fluid, embedded in paraffin, and stained by the usual methods were used to supplement the supravital studies.

The fundamental experiments dealing with giant cells were carried out on the blood and tissues of rabbits which had been injected with various substances, namely, agar, lycopodium spores, paraffin, olive oil, mineral oil, living tubercle bacilli, and certain lipoid fractions isolated from tubercle bacilli. These latter substances were obtained from Dr. R. J. Anderson of the Sterling Chemical Laboratories of Yale University, through the Research Committee of the National Tuberculosis Association. A full account of these reactions has been presented by Doan, Sabin and Forkner (1). None of the substances studied produced giant cells in the circulating blood, except agar. Consequently the development of this special phase of the giant cell study is here presented separately.

Experimental Data

The early experiments in which relatively small amounts of agar were introduced into the tissues produced no significant changes either quantitative or qualitative in the cells of the blood. However, since the local tissue response was so profound such large numbers of monocytes and giant cells having been produced, the possibility presented itself that a larger and more disseminated foreign body reaction might carry over the tissue response to the blood. This theory seemed to have merit since it was known from experience in this laboratory that certain diseases, notably tuberculosis, are often associated with the finding of pathologic tissue elements, epithelioid cells, in the circulating blood.

Accordingly, an animal, R 790, was injected, on February 25, 1929, with plain sterile agar in many areas under the skin and intraperitoneally.

A total of 40 cc. of the agar at pH 7.4 was introduced. Incidentally this animal was given frequent intravenous injections of trypan blue in order that the reactions of the giant cells of the tissues to this vital dye might be studied. Their responses are discussed elsewhere (1). Let it suffice to say that the trypan blue did not materially influence the blood picture in this animal, except that it may have been responsible for the increased number of macrophages.

The blood had been examined prior to the injections, but was not studied again

until 1 week later (March 4). At this time there was a slight reduction of the hemoglobin and number of red corpuscles. The total white blood cell count was not changed. Monocytes were distinctly high (21 per cent) and the lymphocytes were lower than they had been in the control period. The blood platelets were clustered in large masses. Some individual blood plates were larger than normal. The platelets were undoubtedly greatly increased in number. The blood contained 11 per cent of clasmatocytes (macrophages) which were usually large cells with single, centrally placed nuclei. Their cytoplasm contained débris of various shades of color and various sizes and shapes. The presence of the large number of these cells on this occasion may be explained as the result of the injection of trypan blue. Such phenomena have been studied and reported by Simpson (8). The character of the monocytes on this and subsequent days was of great interest. Many of them were much larger than normal, possessed round or oval nuclei, often with two or three nucleoli, and each cell contained a rosette of exceedingly fine uniform neutral red bodies. In some instances the rosettes were small, whereas in others they were hypertrophied, making a stimulated monocyte which is a transition between a normal monocyte and an epithelioid cell. These forms are entirely similar to the developing monocytes which Forkner (27) has described and illustrated as normal elements in peripheral lymph nodes of rabbits. Another interesting change was noted in the polymorphonuclear neutrophils. Many of these cells had a decreased number of specific granules and contained more neutral red bodies than are normally present. These neutral red bodies normally are round or globular whereas after agar injections the same bodies are present and in addition many larger, often elongated, non-refractive vacuoles or granules.

Besides these changes in the usual blood cells, typical multinucleated giant cells could be found in every smear on this and subsequent days until the animal was autopsied on March 18, 1929, 3 weeks after the agar injection. The photograph of one of these cells is illustrated in Fig. 11. This cell had about 25 nuclei scattered through all parts of the cytoplasm. There was an area near the center of the cell which was probably agar. It was a homogeneous amorphous material which had a purple tint. It had apparently taken on some of the trypan blue as the agar masses in the tissue had done. The nuclei near the periphery tended to be oval, whereas the others were round in shape. Individual nuclei were quite uniform in size and were somewhat larger than red blood corpuscles. Some of them contained nucleoli. The cytoplasm contained scattered, irregular masses of material staining all shades of red, yellow, and brown. There was no pattern to the arrangement of the material stained with neutral red.

Fig. 9 represents a cell from the peripheral blood of this animal. A coarse mass of agar is seen to lie across the center of the cell and on either side are segregation bodies entirely similar to those encountered in epithelioid cells and monocytes. One can, in fact, make out two areas in which the arrangement of the vacuoles suggests rosettes of neutral red bodies in the upper part of this cell, indicating a possible fusion of two or more mononuclear or polynuclear cells around the large

mass of agar. This last cell (Fig. 9) is difficult to classify in one or the other group, but on the whole it is more like a foreign body giant cell.

Another interesting group of cells from Rabbit R 790, shown in Fig. 4, illustrates how the giant cells may be formed or how they may be disintegrated. In this figure are three stimulated monocytes which have fused together about a blue mass of agar. When first seen this group had an almost spherical cell membrane surrounding the three cells and the agar. They were observed in the warm box for about 2 hours and were seen to separate into three distinct living cells with only a thread of cytoplasm connecting them. The agar was left free between them. This phenomenon may represent the fate of foreign body giant cells by disintegration into separate uninuclear cells. Other instances have been observed where giant cells of the foreign body type could be seen to be composed of many individual monocytes with other monocytes or epithelioid types nearby, apparently fusing to form a giant cell (Figs. 12 and 13). It would appear then that this is evidence similar to that recorded by Hektoen (13). He studied healing, nondegenerative tuberculous tissue of the brain and found that many giant cells are disintegrated into mononuclear cells which retain their function. It is not clear from his text whether the giant cells which he observed as separating into mononuclear elements were according to the classification here employed "epithelioid giant cells" or foreign body giant cells. I have found no evidence for this phenomenon in "epithelioid giant cells." Later Hektoen (28) supported his former view by observing the same phenomenon in the fate of the giant cells which form in the absorption of coagulated blood serum, in the anterior chamber of the rabbit's eye. In these latter experiments it seems that he was probably dealing with the foreign body type of giant cells.

Rabbit R 579 was injected with agar as the previous animal had been, but no dye was introduced.

A preliminary study of the blood before the injection showed a normal formula. There were 7,600 white blood cells and 5,250,000 red blood cells per cubic millimeter. There were 11 per cent monocytes. 2 days after the injection of agar, the monocytes rose to 20 per cent and increased in total number from 836 to 1,580 per cubic millimeter. 6 days after the injection, monocytes became more numerous than any other cell, increasing to 49 per cent or 5,120 cells per cubic millimeter. A chart (Chart 1) makes this reaction clear. In this experiment, there was a reciprocal relationship between the numbers of monocytes and lymphocytes. As in the preceding experiment, many of the monocytes were of the young type, larger than the adult cells, possessing round or oval nuclei with nucleoli and having an extraordinary abundance of delicate mitochondria. Some of the mature monocytes contained coarse irregular brownish bodies in their cytoplasm, which were probably pieces of agar undergoing digestion.

Giant cells were seen in the blood on the eighth and ninth days. On the eighth day enough were present to be recorded in the differential count on a percentage

basis; on the ninth day, only an occasional cell in an entire film, indicated on the chart by a + sign, was seen. One of these was drawn (Fig. 6). It is clearly of the foreign body type with scattered nuclei and abundant irregular masses of material in the cytoplasm, probably agar undergoing fragmentation and disintegration. The nuclei contained definite nucleoli. Mitochondria were present and easily seen near the periphery of the cell. Adjoining this cell but not fused with it is a monocyte containing the same kind of material as in the giant cell. It is apparent that the larger cell might easily be considered as representing a fusion of many of the individual monocytes. Another cell was drawn (Fig. 7) which has a central rosette and three peripheral nuclei. The material in the cytoplasm is

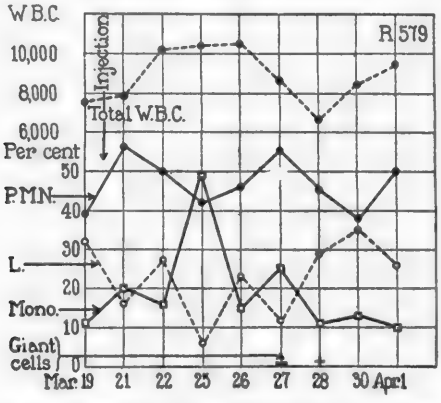

CHART 1. Graph of the white blood cells of Rabbit R 579. The arrow indicates the time at which 40 cc. of sterile plain agar was given in many subcutaneous areas and intraperitoneally. For giant cells the symbol + means the presence of an occasional giant cell in an entire preparation; the solid blocks indicate their percentages when sufficient to appear in the differential count.

not typical of an epithelioid cell. It looks much more like a small foreign body giant cell. It is impossible to say whether this giant cell has arisen by fusion of independent elements or has formed by division of the nuclei. Fig. 8 represents a small foreign body giant cell of the same type as Fig. 7, but containing much coarser bodies. The nuclei are flattened out against the cell wall. This cell, together with that shown in Fig. 7, illustrates that one can not always identify this type of giant cell by the peripheral arrangement of the nuclei. In order to differentiate them properly one must study all of the cellular organs. The presence of a central rosette of fine neutral red bodies aids materially and is probably the most important single factor in the separation of "epithelioid giant cells" or the so-called Langhans' cell. The lungs of this and the other animals were studied in

TABLE I

Changes in the Blood Cells as the Result of Agar Injections into the Tissues

Experiment	Date	White blood cells per cu. mm.	Red blood cells per cu. mm.	Neutrophils		Basophils		Eosinophils		Monocytes		Lymphocytes		Giant cells		Clasmatocytes		Unclassified		Hemoglobin per cent
				Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	
R 790	1/12/29	4,850	5,490,000	45	2,182	5	242	1	48	11	533	38	1,843							68
	1/14	6,400	5,800,000	30	1,920	14	896	2	128	7	448	47	3,008							65
	2/25	6,250	6,200,000	56	3,500	8	500	1	63	7	438	28	1,750							76
	2/25	Began frequent intravenous injections of trypan blue. Injected 40 cc. of agar intraperitoneally and subcutaneously.																		
	3/4	5,500	4,580,000	52	2,860	6	330			21	1,155	10	550		+	11	605			57
	3/6	4,100	4,000,000	58	2,378	14	574	1	41	6	246	18	738		+	3	123			56
	3/7	7,500	4,140,000	63	4,725	10	750	1	75	2	150	22	1,650		+	1	75	1	75	55
	3/9	3,800	4,360,000	62	2,356	12	456	1	38	5	190	19	722		++					49
	3/11	4,200	4,940,000	72	3,024	1	42	2	84	1	42	23	966		+	1	42			56
	3/12	7,950	4,480,000	72	5,724	2	159	2	159	3	238	21	1,669		+					61
	3/15	8,300	3,790,000	68	5,644	18	1,494	1	83	4	732	8	664		+	1	83			53
	3/18	7,250	4,690,000	53	3,842	22	1,595			8	580	13	942		+	2	145	2	145	62
R 579	3/19	7,600	5,260,000	39	2,964	17	1,292	1	76	11	836	32	2,432							
	3/19	Injected in 6 subcutaneous areas intraperitoneally with 40 cc. of plain agar.																		
	3/21	7,900	4,840,000	56	4,424	5	395			20	1,580	16	1,264					3	237	52
	3/22	10,200	4,540,000	50	5,100	4	408	2	204	16	1,632	27	2,754					1	102	55
	3/25	10,450	5,260,000	42	4,389	1	104			49	5,120	6	627					2	209	64
	3/26	10,550	5,210,000	46	4,853	10	1,055	4	422	15	1,582	23	2,426					2	211	59
	3/27	8,700	5,320,000	55	4,785	3	261			25	2,175	12	1,044	1	87			4	348	64
	3/28	6,700	4,620,000	45	3,015	12	804	2	134	11	737	29	1,943	++	++			1	67	58
	3/30	8,450	4,520,000	38	3,211	13	1,098	1	85	13	1,098	35	2,957							
	4/1	9,500	4,790,000	50	4,750	12	1,140	2	190	10	950	26	2,470							55

R 580	3/19	11,000	4,280,000	37	4,070	9	990	1	110	9	990	43	4,730					1	110	60
	3/19	4 cc. trypan blue every 2nd day and 40 cc. plain agar injected intraperitoneally and subcutaneously today.																	
	3/21	45,500	4,440,000	52	23,608	4	1,816			37.5	17,025	4.5	2,043		+			2	908	52
	3/22	21,900	4,630,000	53	11,607	1	219			40	8,760	4	1,314							65
	3/23	42,850	4,300,000	41	17,569	1	429			38	16,283	11	5,994		+	1	429	5	2,142	
	3/25	19,050	3,920,000	49	8,954	2	381	2	381	28	5,905	8	1,524		+	1	191	9	1,715	55
	3/26	24,950	4,170,000	62	15,469	3	749			11	2,745	8	2,745		+			13	3,244	58
	3/27	5,400	4,520,000	56	3,024	9	486	4	216	14	864	15	810		+					60
	3/28	4,400	4,320,000	55	2,420	7	308			14	660	20	968	1	44					52
R 967	3/16	Injected intravenously with 1 mg. T. B.																		
	3/25	5,300	6,150,000	52	2,756	8	424	1	53	4	212	34	1,802					1	53	62
	3/28	Injected subcutaneously and intraperitoneally with 40 cc. trypan blue every 2nd day.																		
	3/29	5,050	5,270,000	77	3,889	7	353			6	303	8	404							52
	3/30	6,150	4,780,000	42	2,583	3	185			42	2,583	9	553	+	+			4	246	54
	4/1	5,400	5,240,000	29	1,566	5	270	1	54	41	2,214	15	810	4	216	5	270			52
	4/3	7,350	5,850,000	24	1,764	11	808			39	2,866	19	1,397		++	3	220	4	294	54
	4/5	5,000	5,060,000	34	1,700	9	450			34	1,700	14	700	5	250			4	200	62
	4/6	5,150	5,110,000	65	3,347	8	412			17	875	10	515							54
	4/8	6,400	5,520,000	51	3,264	6	384	1	64	12	768	17	1,088	7	448	1	64	5	320	56
	4/9	5,800	5,260,000	61	3,538	5	290			13	754	19	1,102	2	116					56
	4/10	18,300	4,820,000	71	14,823	1	183			14	2,562	4	732		++					64
	4/11	5,950	5,260,000	64	3,808	3	178			11	655	14	833	3	179	4	238	1	60	53
	4/12	3,850	5,280,000	40	1,540	6	231			33	1,270	7	270	3	115	5	193	6	231	55
	4/13	12,500	5,020,000	77	9,625	4	500			9	1,125	7	875	1	125	1	125	1	125	52
	4/15	8,950	4,790,000	81	7,339	3	269			9	806	3	269	2	179	1	89			52
	4/16	6,850	4,020,000	77	5,275	5	342			10	685	5	342	1	69	1	69	1	69	44

TABLE I—*Concluded*

Experiment	Date	White blood cells per cu. mm.	Red blood cells per cu. mm.	Neutrophils		Basophils		Eosinophils		Monocytes		Lymphocytes		Giant cells		Clasmatocytes		Unclassified		Hemoglobin per cent
				Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	
R 939	5/20	8,450	4,610,000	49	4,140	4	338			23	1,943	22	1,859					2	169	
	5/20	Injected intraperitoneally and subcutaneously with about 40 cc. sterile plain agar containing 10 cc. of India ink.																		
	5/21	26,100	3,690,000	76	19,836	2	522	3	783	14	3,654	5	1,305							
	5/22	18,850	3,810,000	60	11,310					25	4,712	14	2,639		+			1	188	
	5/23	10,300	4,490,000	65	6,695	1	103			27	2,781	7	721							
	5/24	14,850	4,680,000	50	7,425	4	594			23	3,415	19	2,821	3	445			1	148	
	5/27	6,750	4,620,000	47	3,172	3	202			24	1,620	22	1,485	4	270					
	5/29	7,650	4,330,000	67	5,125	2	153			11	841	20	1,530		+					
	5/31	6,500	4,640,000	65	4,225	2	130	3	195	8	520	22	1,430		++					
	6/3	10,100	4,910,000	80	8,080	1	101			2	202	16	1,616		+			1	101	
	6/5	8,750	4,200,000	92	8,050					4	350	3	262					1	87	
	6/6	8,150	4,930,000	86	7,009	8	652			2	163	4	326							

sections. Numerous giant cells were easily seen in the smaller capillaries. Fig. 14 illustrates one of the giant cells in the lung of this animal.

Rabbit R 580 received the same type of injections as R 790.

Trypan blue (1 per cent solution in distilled water) was given intravenously or intraperitoneally in 4 to 10 cc. doses every second day and in addition the animal re-

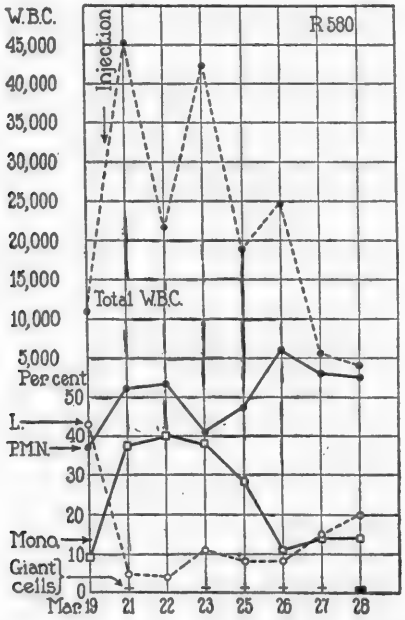

CHART 2. Graph of the white blood cells of Rabbit R 580. The arrow indicates the time at which 40 cc. of sterile plain agar was introduced subcutaneously and intraperitoneally. At this point injections of trypan blue were begun and were continued on every second day.

ceived plain agar into many areas of the subcutaneous tissue and peritoneal cavity. Prior to the injections the cells of the blood were entirely normal (Table I, Chart 2). There were 11,000 leucocytes per cubic millimeter. However, on the day following, the total leucocyte count rose to 45,500 cells per cubic millimeter—an increase of over 400 per cent. This rise was chiefly brought about by the monocytic strain of cells. The neutrophiles also increased somewhat, but the lymphocyte curve descended, their number being represented by only 4 per cent of the total white

blood cells. At the height of the reaction there were over 17,000 monocytes in each cubic millimeter of blood. Many of these monocytes were of the young type previously described. Some of the monocytes and neutrophiles appeared to contain fragments of agar. Many of the neutrophiles had lost a considerable portion of their specific granules. There was a tendency for the leucocytes to be clumped in large masses. Some of the young monocytes contained two oval nuclei.

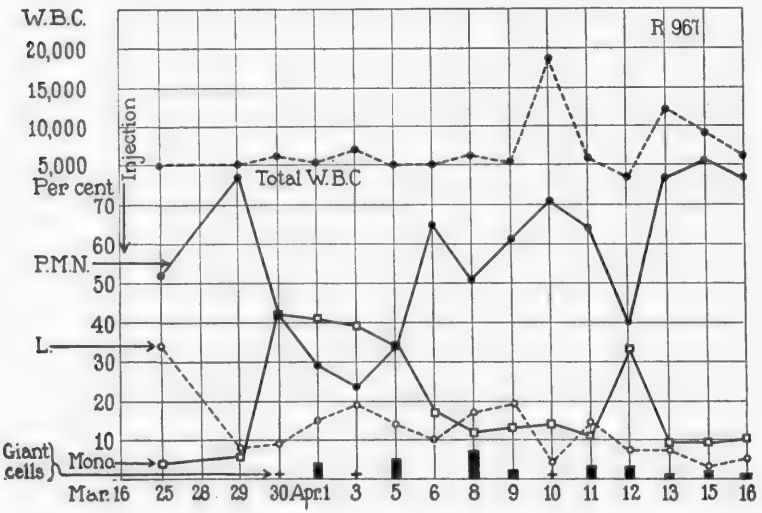

CHART 3. Graph of the white blood cells of Rabbit R 967. The first arrow shows the time at which the rabbit received 1 mg. of avirulent tubercle bacilli intravenously. This presumably did not modify any of the changes found in the chart. The second arrow indicates the time at which 40 cc. of sterile plain agar was administered subcutaneously and intraperitoneally. At this time also injections of trypan blue were begun and they were continued every second day.

2 days after the agar injection, the total leucocyte count was 21,900 per cubic millimeter, but the percentage of monocytes was even higher (40 per cent). The accompanying table and chart (Table I, Chart 2) will make these changes clear. 3 days after the beginning of the experiment the total white blood cell count was again elevated to 42,850 cells per cubic millimeter, but there were no significant changes in the percentages of cells except a rise of lymphocytes from 4 to 11 per cent. Giant cells of both types began to appear in the blood and could be found in practically every smear thereafter. 6 days after the injection, a mild leucopenia set in with the monocytes constituting about 14 per cent of the cells.

Rabbit R 967 was interesting because it showed giant cells in large numbers in the blood stream.

On March 16, 1928, the animal received an intravenous injection of 1 mg. of avirulent tubercle bacilli which did not produce progressive tuberculosis and which presumably caused no significant changes in the blood picture to be described. The blood picture was normal on March 25 when intravenous injections of trypan blue were begun and continued every second day for nine doses. This may account for the presence from time to time of from 3 to 5 per cent of clasmatocytes (macrophages) in the blood. The animal was injected with plain agar in a number of areas on March 28. The chart and table (Table I, Chart 3) show the changes in the total number and percentage values of the cells. The chief result of the injection on the blood was a rather striking and prolonged rise in monocytes together with a maintained decrease in the percentage and absolute number of lymphocytes.

This animal differed from the preceding one in that there was no sharp rise in total leucocytes within a short time after the injection. However, there did occur the marked elevation of percentage of monocytes. This increase was found on the second day and persisted for 1 week during which time the monocytes ranged from 34 to 42 per cent of the circulating white blood cells. During the next 2 weeks monocytes remained abundant, varying between 9 and 33 per cent. Giant cells occurred in the blood 2 days after the agar injection and were found in every film of blood until autopsy 17 days later. They frequently were so numerous as to constitute 2 or 3 per cent of the total number of leucocytes and on one occasion reached as high as 7 per cent. It was not uncommon to find two in a single high power field of the microscope. Most of the "epithelioid giant cells" contained from 2 to 7 nuclei (Fig. 15) but an occasional one had from 10 to 14 nuclei; the foreign body giant cells, on the other hand, contained from 2 to 50 nuclei, many of them having from 15 to 30.

Fig. 2 illustrates what might be called a mononuclear giant cell of the blood. It measured 26.7 by 31.4 microns, about twice the size of an ordinary monocyte. It had a large indented nucleus with a nucleolus, a definite rosette of fine neutral red bodies, together with numberlesss delicate mitochondria. This cell would fall into the monocyte group and would be further qualified by saying that it is a young stimulated form. If the rosette were slightly larger we would call it an epithelioid cell. 4 days after the injection, when monocytes constituted 41 per cent of the white blood cells, this type of cell, the young, large, stimulated form, was very abundant. Cells entirely identical with this except for the presence of 2, 3, or more nuclei were frequently found. Fig. 3 illustrates one of these epithelioid giant cells with 9 nuclei. All intermediate stages between the cells represented by Figs. 2 and 3 could readily be demonstrated in the blood.

At first the mechanism by which the giant cells reached the peripheral veins was not understood for some of these cells were more than

50 microns in their smallest diameters. It is difficult to conceive how these cells could find their way through the capillaries of the lungs and then into the systemic circulation. Figs. 5 and 10 illustrate how this phenomenon is accomplished. The cell in Fig. 10 was taken from the peripheral ear vein of Rabbit R 967 and shows that these giant cells are not rigid but that they may become elongated to such an extent as to pass through very small capillaries. Fig. 5 was from the

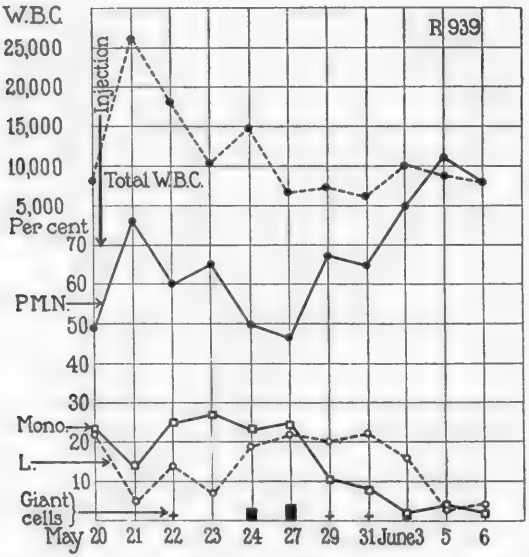

CHART 4. Graph of the white blood cells of Rabbit R 939. The arrow indicates the time at which 40 cc. of sterile plain agar was injected into the subcutaneous tissues and into the peritoneal cavity.

central artery of the ear of Rabbit R 939 and illustrates the apparent ease with which the cells may make their way through the capillary bed.

The next experiment which was tried was to mark the agar in some way in order that it might be identified when it was transported within cells and to distant parts of the body. This was carried out by adding to the 40 cc. of agar to be injected 10 cc. of Higgins' India ink. This was mixed carefully and made an intensely black preparation.

Rabbit R 939 was chosen for the experiment. The count prior to the injection showed the animal to have 23 per cent, an abnormally high value, of monocytes (Table I, Chart 4). On the day following the injection, the total leucocytes rose to 26,100 cells per cubic millimeter. The monocytes rose and remained high for several days. Giant cells of both types appeared in very small numbers in the blood on the second day and were found on several occasions over a period of 2 weeks. Occasionally a few granules of carbon could be seen in the giant cells, but not as much as was expected. Fig. 1 shows an epithelioid giant cell taken from the arterial blood, which has several definite fragments of agar containing carbon. The autopsy on this animal demonstrated that the carbon had not been as evenly distributed in the agar as was expected. The giant cells immediately adjacent to the ink deposits contained it in great abundance, but most held relatively little.

Many more animals were subjected to similar experiments with agar and with other substances. It was not possible to induce regularly an appearance of cells in the blood with any of the substances used except agar. In one animal which had been injected with large amounts of lycopodium spores, one giant cell was found in the blood on a single occasion. This finding could not be repeated.

DISCUSSION

These studies on the peripheral blood have made it possible to form definite ideas about the origin and development of "epithelioid giant cells" and of foreign body giant cells. It becomes necessary to consider these elements histologically as two groups of cells which have a common origin from monocytes. Under the conditions of these experiments it appears that the great majority of foreign body giant cells are formed by the fusion of monocytes, and that the majority of "epithelioid giant cells" are formed by amitotic division of the nucleus or nuclei of epithelioid cells which latter elements are monocytes of large size, with hypertrophied rosettes. The results of the studies would thus confirm the theories of both schools of pathologists, of those who consider that giant cells are the products of fusion, and those who maintain that they form by division of the nuclei. The difference of opinion on this point has resulted doubtless from the failure to recognize that in almost every tissue reaction which produces giant cells, both types are present, but that their relative numbers assume different proportions according to the character of the

substances which excite the response. No evidence has been observed in these studies to support the theory that epithelioid giant cells are formed by the fusion of individual cells. On the other hand, many epithelioid cells and "epithelioid giant cells" with partially divided nuclei, and others which appeared as though the nuclei had recently divided amitotically have been seen.

Regarding the fate of giant cells, no definite conclusions are possible but these studies lend support to the conclusion of Hektoen (28) that foreign body giant cells may separate into their constituent elements. No evidence is available that "epithelioid giant cells" undergo a similar process.

The occurrence of the two types of giant cells in the circulating blood may be explained in either of two ways. They may be formed in the blood vessels in the neighborhood of the foreign body reaction, or they may form extravascularly and migrate into the vessels. The latter explanation would appear more tenable, for giant cells are to some extent motile, and can become so elongated that passage through the capillary walls should be entirely possible. Furthermore, it would be difficult to explain the presence of large pieces of agar in the cells, as shown in Figs. 4 and 9, by any other hypothesis than that they have been carried within cells from the tissues into the blood.

SUMMARY AND CONCLUSIONS

1. It has been demonstrated that giant cells of the foreign body and epithelioid types can be induced to appear in the blood stream.

2. Evidence has been presented which indicates that foreign body giant cells are primarily formed by fusion of monocytes and that the fate of these giant cells is accomplished, at least in some instances, by a separation into the constituent elements.

3. Further evidence has been presented which lends support to the hypothesis that "epithelioid giant cells" reach their stage of evolution, not by fusion of monocytes, but by amitotic division of the nuclei of monocytes and epithelioid cells.

4. The presence of giant cells in the peripheral blood as the result of agar injections is almost invariably associated with, or preceded by a marked monocytosis in which the new monocytes are of large size and show evidence of immaturity.

5. Injections of agar into the tissues result in decreased absolute and percentage values of lymphocytes and a diminution of the specific granules in many of the polymorphonuclear leucocytes.

6. It would appear from these studies that a clear differentiation of "epithelioid giant cells" and "foreign body giant cells" in the blood is usually possible, but that on the other hand, a few cells may be present which have some of the characteristics of each type. These latter cells probably represent in their formation both a fusion of individual cells and an amitotic division of the nuclei of monocytes.

7. Clasmatocytes or macrophages have in rare instances been seen to take part in the formation of foreign body giant cells. At least one instance has been noted of the fusion of a clasmatocyte with several monocytes. No evidence is available to demonstrate that macrophages ever play a part in the formation of "epithelioid giant cells."

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EXPLANATION OF PLATES

PLATE 10

Drawings of cells from arterial and venous blood of rabbits after injections of agar; stained with neutral red and Janus green and drawn while still alive.

FIG. 1. An epithelioid giant cell from the peripheral arterial blood of Rabbit R 939. Small aggregations of carbon can be seen. The mitochondria are very abundant and are best seen at the periphery of the rosette. This cell measured 39.3 x 33 microns.

FIG. 2. A huge, young, stimulated monocyte from Rabbit R 967. It is almost as large as the cell in Fig. 1, but contains only a single nucleus with a large nucleolus. Thousands of delicate mitochondria are stained with Janus green and a typical dense rosette is stained with neutral red. Cells identical with this, except for the presence of two or more nuclei, could be found in practically every field. This cell measured 31.4 x 26.7 microns.

FIG. 3. Epithelioid giant cell from venous blood of Rabbit R 967, with nine nuclei. It has a unified central rosette, similar to those of Figs. 1 and 2. Size 36.1 x 33 microns.

FIG. 4. Three monocytes which have fused around a piece of agar in the blood of Rabbit R 790. This animal had received trypan blue intravenously on several occasions and the agar foci were stained in an identical manner, as illustrated in this figure. When first seen this cell was practically spherical, but after 2 hours the three constituent cells were almost entirely independent of each other. Size 35 microns at widest diameter.

FIG. 5. This huge giant cell was obtained from a peripheral artery of Rabbit R 939. It measured 107 microns in length. It illustrates very well that these cells are capable of changes in shape so great as to enable them to pass through the capillaries. A red blood corpuscle is shown to give an idea of their great sizes.

FIG. 6. A foreign body giant cell with many nuclei from the venous blood of Rabbit R 579. This cell and the monocyte nearby are filled with masses of irregular, fragmented material. This material is probably agar undergoing changes within the cell. Size 67.5 × 47.1 microns.

FIG. 7. A trinucleated cell containing the same general type of material as that in the cell of Fig. 6. The mitochondria have faded somewhat. This cell is also from the blood of Rabbit R 579. Size 21 microns in diameter.

FIG. 8. Another foreign body giant cell from the blood of the same animal. Size 28 × 17 microns.

FIG. 9. A foreign giant cell from the blood of Rabbit R 790. There is a large, lobulated mass of material in the center of the cell which is probably agar that has not as yet undergone very much change within the cell. In the upper left region of the cell two rosettes can be seen. Size 84 x 52.5 microns.

FIG. 10. Another elongated cell. This foreign body giant cell was from the blood of Rabbit R 967. Size 73.8 by 14 microns.

PLATE 11

Four photographs of living cells supravitally stained with neutral red and Janus green, and one photograph (Fig. 14) from a section.

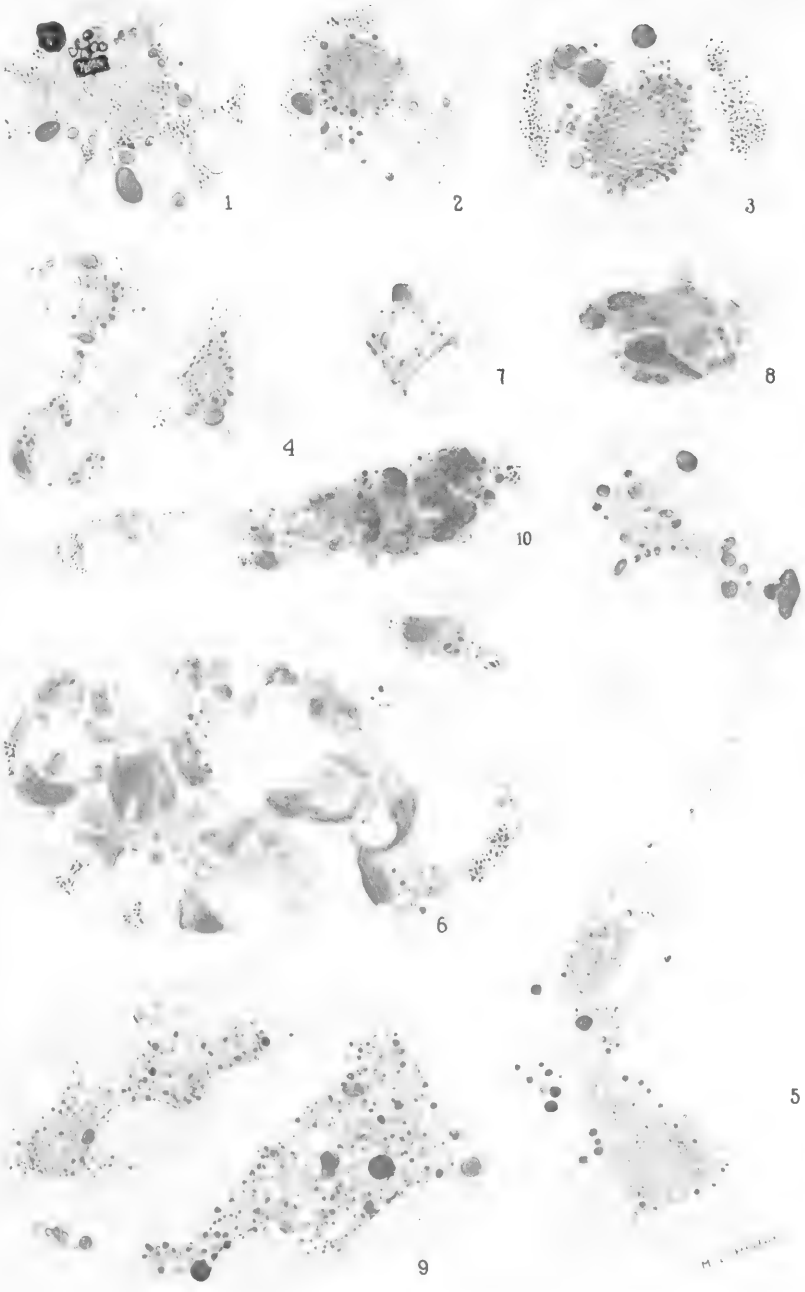
FIG. 11. Photograph of a large foreign body giant cell from the blood of Rabbit R 790. It has very many nuclei scattered throughout the cell and the cytoplasmic bodies are arranged in no definite pattern. Several monocytes can be seen on the surface of the cell, as if in process of fusion. Red blood corpuscles can be seen for comparison of size. Magnification × 575. Stained with neutral red in supravital preparation.

FIG. 12. Photograph of a foreign body giant cell in the blood with many monocytes and epithelioid cells about it. This animal, R 939, had received agar plus India ink which was injected under the skin and into the peritoneal cavity. Note the definite rosettes of neutral red bodies in the monocytes and epithelioid cells. Magnification × 750. Stained with neutral red in supravital film.

FIG. 13. Photograph of an accumulation of monocytes about a foreign body in the blood of Rabbit R 967. The cell at the upper end of the figure is a clasmatocyte. The cytoplasm of these cells could not be separated. They were apparently fused. A single monocyte may be seen nearby at the lower border of the figure. Magnification × 750. Stained with neutral red by means of the supravital method.

FIG. 14. Photograph of a giant cell in a capillary of the lung of Rabbit R 579. Many giant cells could be demonstrated in the lungs of this and other animals similarly treated. Magnification × 750. Stained in fixed section with hemotoxylin and eosin.

FIG. 15. Photograph of an epithelioid giant cell in the blood of Rabbit R 967. Note the unified central rosette and peripheral nuclei. Contrast with Fig. 11. Magnification × 750. Stained with neutral red.



(Forkner: Multinucleated giant cells in blood)

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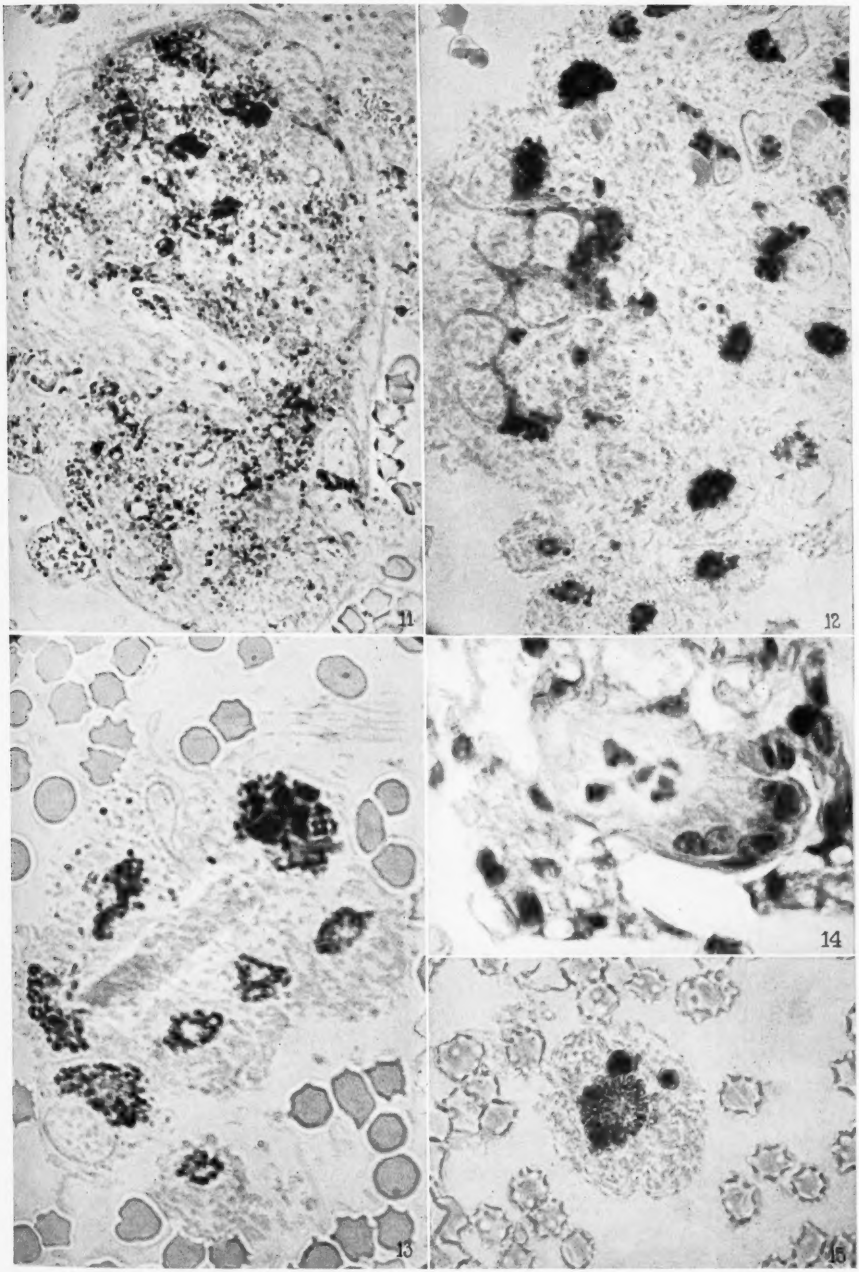

(Forkner: Multinucleated giant cells in blood)

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